

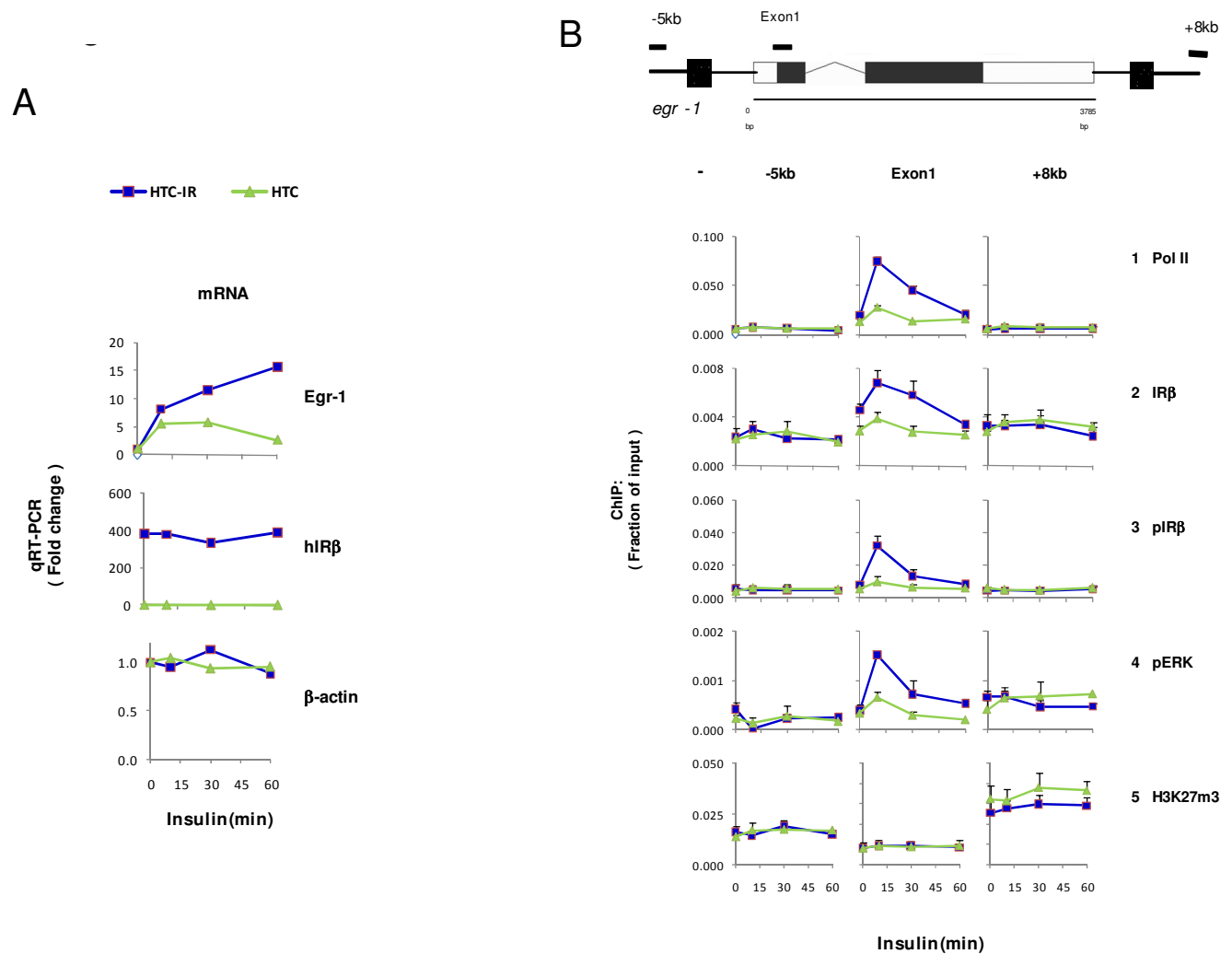
ONLINE APPENDIX - Supplementary material

Direct recruitment of insulin receptor and ERK signaling cascade to insulin-inducible gene loci

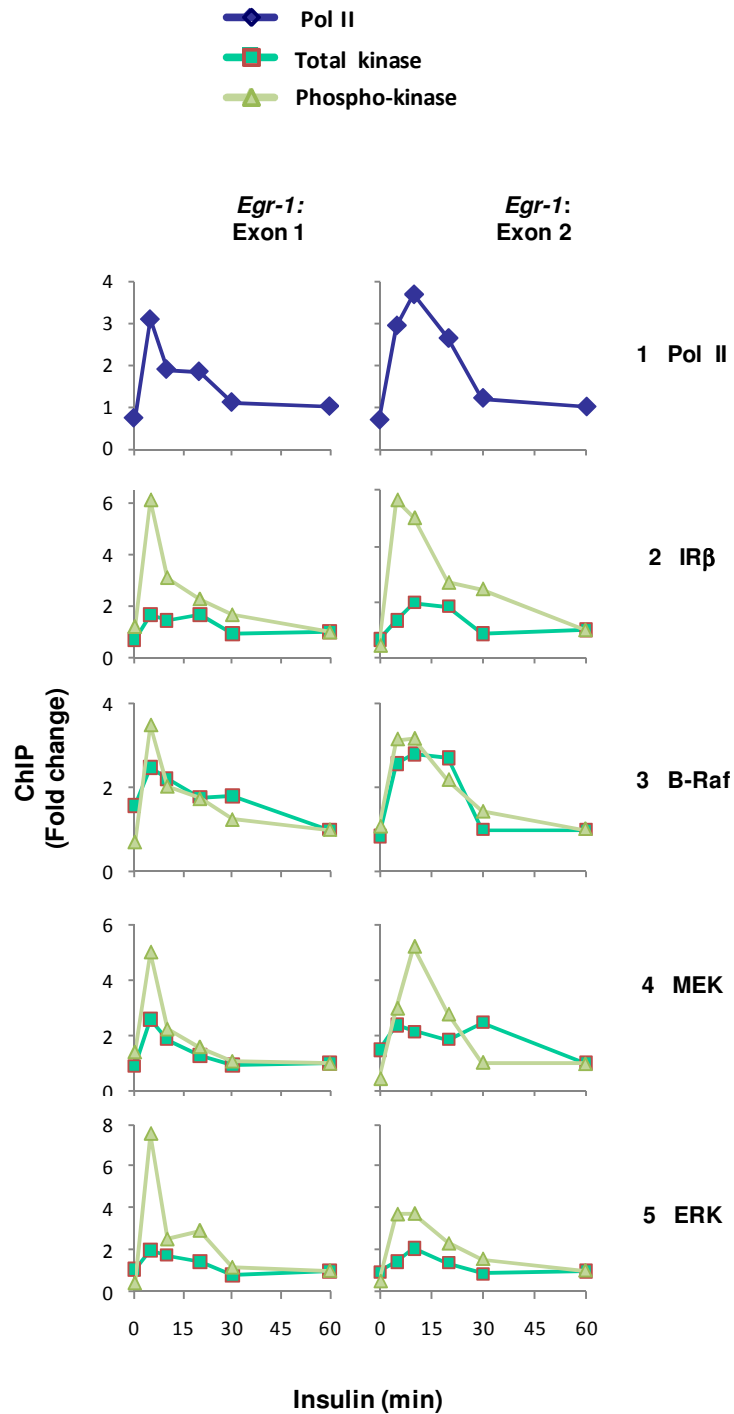
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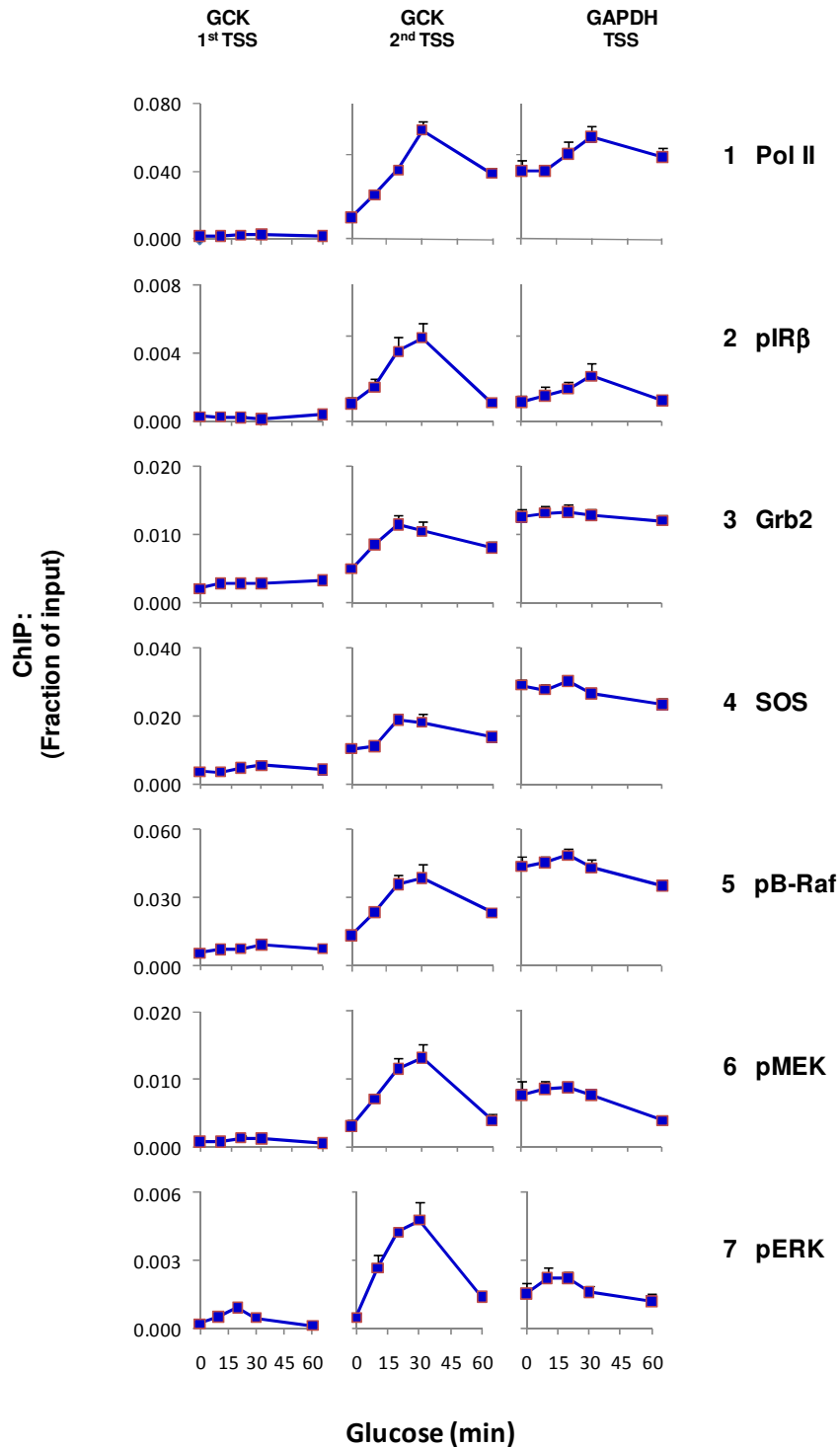
Supplementary Figure 1 – Insulin induced IR and kinase recruitment is specific to cells expressing IR. Serum deprived rat hepatocyte cell line with (HTC-IR) or without (HTC) the human insulin receptor [1] was treated with insulin (10^{-8} M) for 5, 10, 20, 30, and 60 minutes or left untreated. **A.** Total cellular RNA from these cells was used in RT reactions with random hexamers. cDNAs were used in real-time PCR with primers to the last exon of *egr-1*, the human insulin receptor gene, or β -actin. **B.** Cells were treated as in A, cross-linked, and then used in ChIP with antibodies to Pol II, IR β , pIR β , pErk and H3K27m3. ChIPed DNA was used in PCR reactions with primers to -5.3 kb, +232 bp, and +8 kb with respect to the TSS of *egr-1*. A-B, Data represent the mean \pm SEM of two independent experiments.



Supplementary Figure 2 – Synchrony in the kinetics of Pol II recruitment and kinase activation at insulin- induced *egr1*. ChIP data from Figs. 2C and 3C were plotted with total and phosphorylated kinases on the same axes at the +232 bp (Exon1) and +3.1 kb (Exon2) sites of *egr1*.



Supplementary Figure 3 – Specificity of feeding-induced phospho-IR recruitment to the *Gck* locus. ChIP data from figure 4C using primers for the 1st and 2nd TSS of *Gck* was compared to that using primers to the *gapdh* TSS.



Supplementary Table 1 – Primers used in real-time PCR

Rat <i>egr-1</i> , -252 bp	FWD	GCCGGAACAGACCTTATTTG
	REV	GCCTAGTATTGGAACAGCGG
Rat <i>egr-1</i> , +232	FWD	ACCACCCAACATCAGCTCTC
	REV	AGCAGCATCATCTCCTCCAG
Rat <i>egr-1</i> , +3319 bp	FWD	GTCTTGGTGCCTTTGTGTGA
	REV	CCTCTTCCTCATCGTGCTCT
Rat <i>egr-1</i> , -5344 bp	FWD	TCCTCGGGAAGAGAGACAAC
	REV	TACAGTGGACAGATGGCTGG
Mouse β -actin (for RT-PCR)	FWD	GCTGCGTTTTACACCCTTTC
	REV	CTGTGCGCCTTCACCGTTC
Mouse <i>Spot-14</i> (for RT-PCR)	FWD	CGAAACGCTATCCCAAGAAC
	REV	AGCTGCACATCCCTCAGAA
Mouse <i>Fasn</i> (for RT-PCR)	FWD	CCCTGGTGTCTCCTCAGT
	REV	CGACGCCCTCATCTCTGT
Mouse <i>Sdc-1</i> (for RT-PCR)	FWD	AGGTTTCCAAGCGCAGTTC
	REV	TCAGTTGCTCGCCTCACTT
Mouse <i>Foxo1</i> (for RT-PCR)	FWD	GTCTCCCGGTACTTCTCTGCT
	REV	GTGGTCGAGTTGGACTGGTT
Mouse <i>egr-1</i> (for RT-PCR)	FWD	CACCACCCAACATCAGTTCTC
	REV	GCAGCATCATCTCCTCCAGT
Mouse GAPDH TSS	FWD	CCCATCACGTCCTCCATC
	REV	TGGGCACTGTACGGGTCT
Mouse Gck upstream TSS	FWD	GTTTGCATGTCCCCAACAC
	REV	TCCACAGATTGCTACCCACA
Mouse Gck downstream TSS	FWD	TGCAAACTCAGCCAGACA
	REV	GGGCTCCCCTCCTTGTAGT
Mouse Gck intron 3	FWD	TTCTATTTCTGGTCTCCCATCT
	REV	CATACTTGCCCCTCCTTCCT
Mouse Gck 3' end	FWD	GGGGTGGAGGTATATGAAGGA
	REV	CTTGCCTGTGGCTGAAGAG
Mouse L32	FWD	TTAAGCGAAACTGGCGGAAAC
	REV	TTGTTGCTCCCATAACCGATG

Supplementary Table 2 - List of antibodies use in ChIP assays

Antibody	Catalog No	Source	Manufacturer	Immunogen
Pol II CTD (4H8)	sc-47701	mouse monoclonal	Santa Cruz	C-terminal domain repeats(CTD)
IRβ	sc-711	rabbit polyclonal	Santa Cruz	C-terminus
pIRβ(Y1146)	3021	rabbit polyclonal	Cell Signaling	pTyr1146
pIRβ(Y1146)	SA-390	rabbit polyclonal	Enzo	pTyr1146
Grb2	sc-255	rabbit polyclonal	Santa Cruz	C-terminus
SOS1&2	S15530	rabbit polyclonal	Transduction Lab	mouse Sos1 aa 1–109, mouse Sos2 aa 1095–1297
SOS 1&2 (D21)	sc-259	rabbit polyclonal	Santa Cruz	N-terminus
B-Raf (C-19)	sc-166	rabbit polyclonal	Santa Cruz	C-terminus
p-B-Raf	sc-28006r	rabbit polyclonal	Santa Cruz	pThr598/pSer601
Mek1/2	M17030	rabbit polyclonal	Transduction Lab	
Mek1 +Mek2	ab70613	rabbit polyclonal	Abcam	MEK Ser 218/222 and MEK2 Ser 222/226
pMek1/2(S217/221)	9121	rabbit polyclonal	Cell Signaling	Phospho-MEK1 (pSer217/221)
ERK1(K-23)	sc-94	rabbit polyclonal	Santa Cruz	subdomain XI
pErk1/2	9101	rabbit polyclonal	Cell Signaling	pThr202/pTyr204
pErk1/2	sc-101760	rabbit polyclonal	Santa Cruz	pThr202
SRF(G-20)	sc-335	rabbit polyclonal	Santa Cruz	C-terminus
MKP-1(V-15)	sc-1199	rabbit polyclonal	Santa Cruz	C-terminus
Histone H3	ab1791	rabbit polyclonal	Abcam	
H3K9,14Ac	005-044	rabbit polyclonal	Diagenode	
H3K79m2	ab3594	rabbit polyclonal	Abcam	
H3K27m3	ab6002	rabbit polyclonal	Abcam	

1. Iwamoto Y, Wong KY, Goldfine ID (1981) Insulin action in cultured HTC and H35 rat hepatoma cells: receptor binding and hormone sensitivity. *Endocrinology* 108: 44-51.