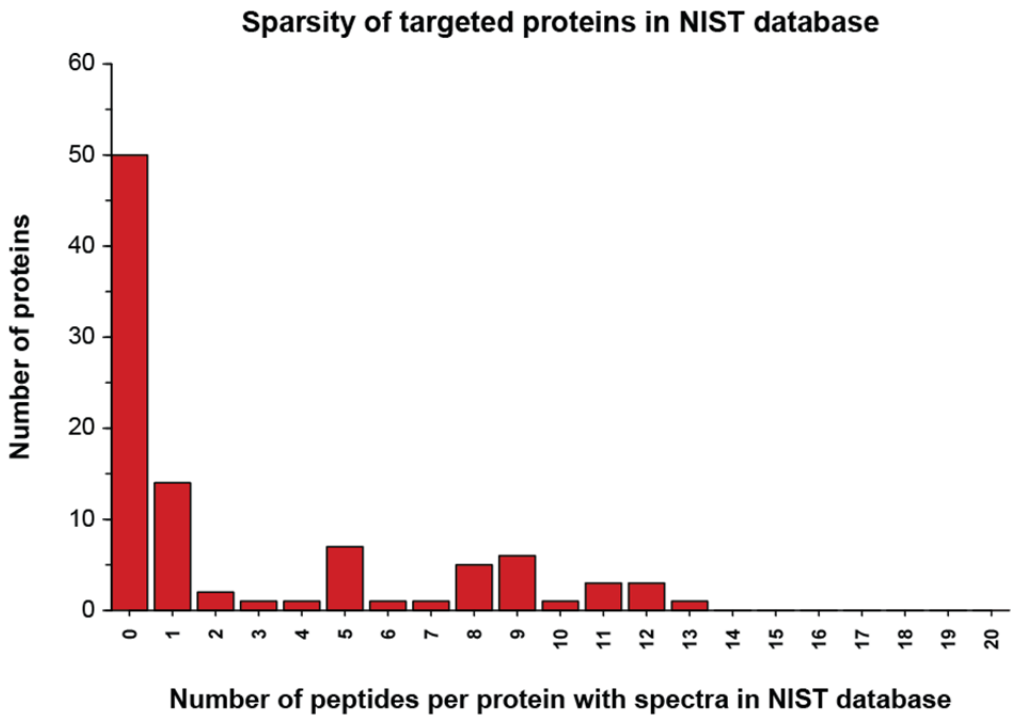
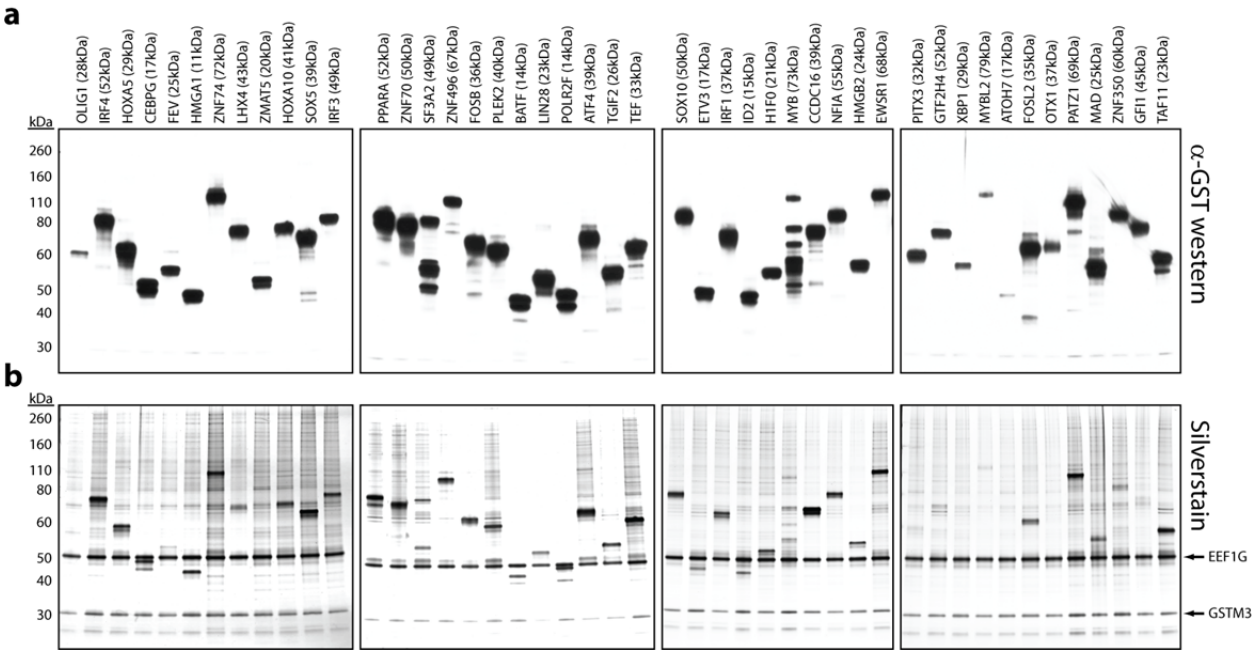
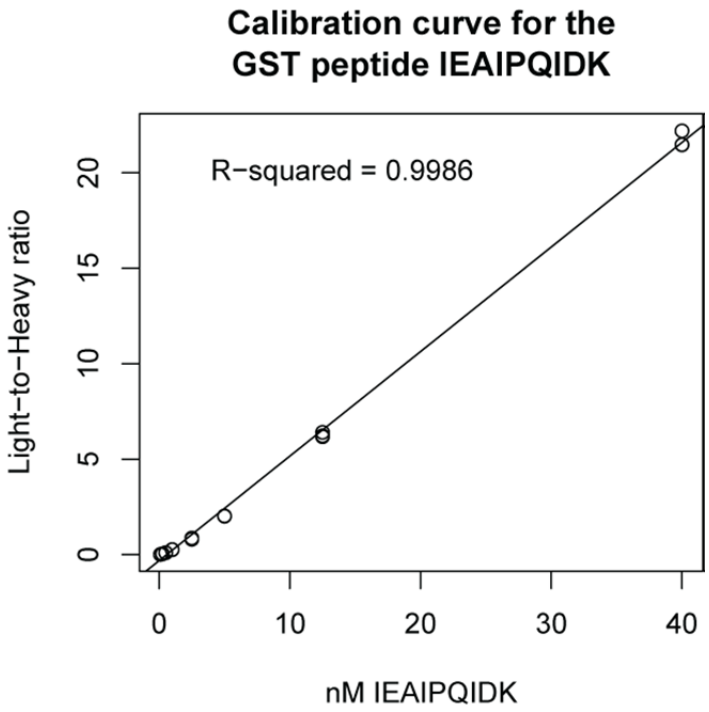


Supplementary Figure 1. Poor coverage of target transcription factor proteins in NIST database

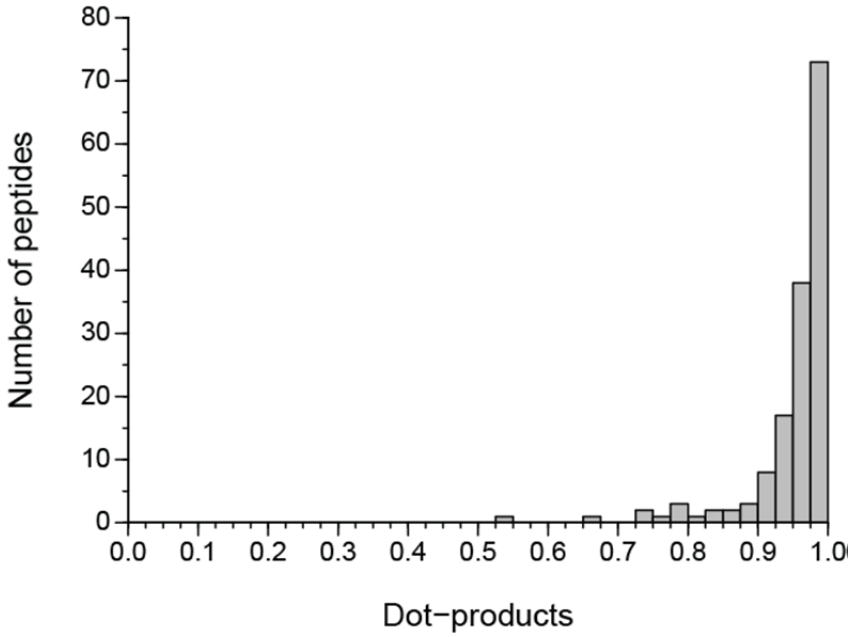
A histogram of the number peptide MS/MS spectra in the NIST database per target protein demonstrates how underrepresented transcription factors are from current peptide spectral libraries. A majority of our target proteins have no MS/MS spectra in the NIST spectrum library.



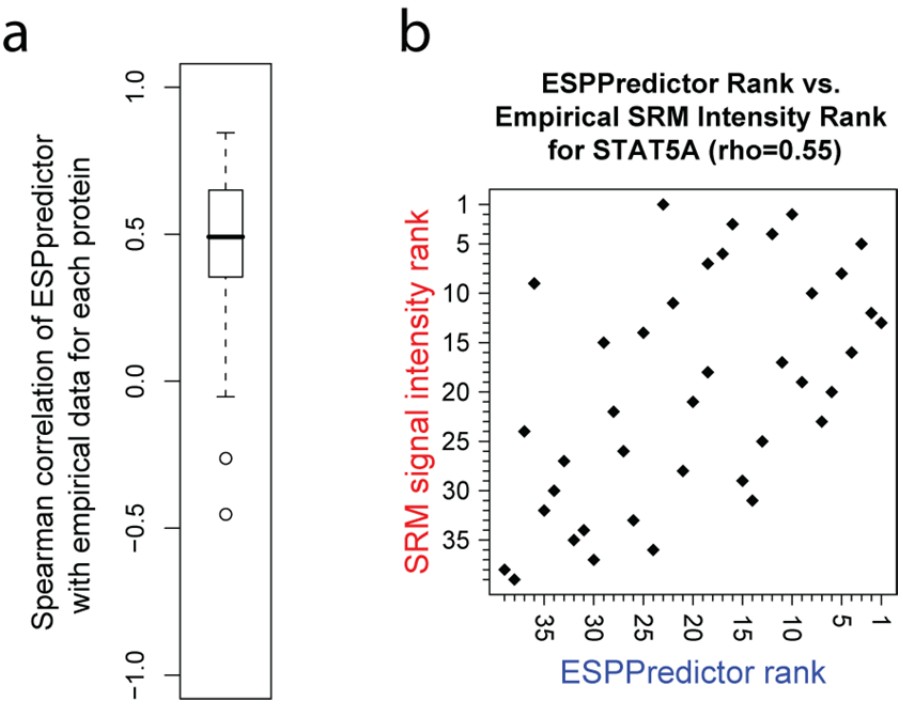
Supplementary Figure 2. ***In vitro*-synthesized proteins are enriched full-length proteins (a-b)** Glutathione-enriched protein samples from 46 of the 96 reactions were run on a denaturing SDS-PAGE gel and subjected to either western blotting with an anti-schistosomal GST antibody **(a)** or silverstaining **(b)**. The endogenous glutathione-binding proteins EEF1G and GSTM3 were identified using 'shotgun' mass spectrometry. The molecular weight of the full length cDNA is indicated in parentheses. These weights do not include the 26kDa GST tag.



Supplementary Figure 3. Calibration curve for the schistosomal GST peptide IEAIPQIDK
A dilution curve of the unlabeled schistosomal GST peptide IEAIPQIDK peptide standard was spiked with a constant amount of the heavy labeled IEAIPQIDK peptide. The unlabeled to labeled peak area was measured by LC-SRM-MS for each standard in triplicate.

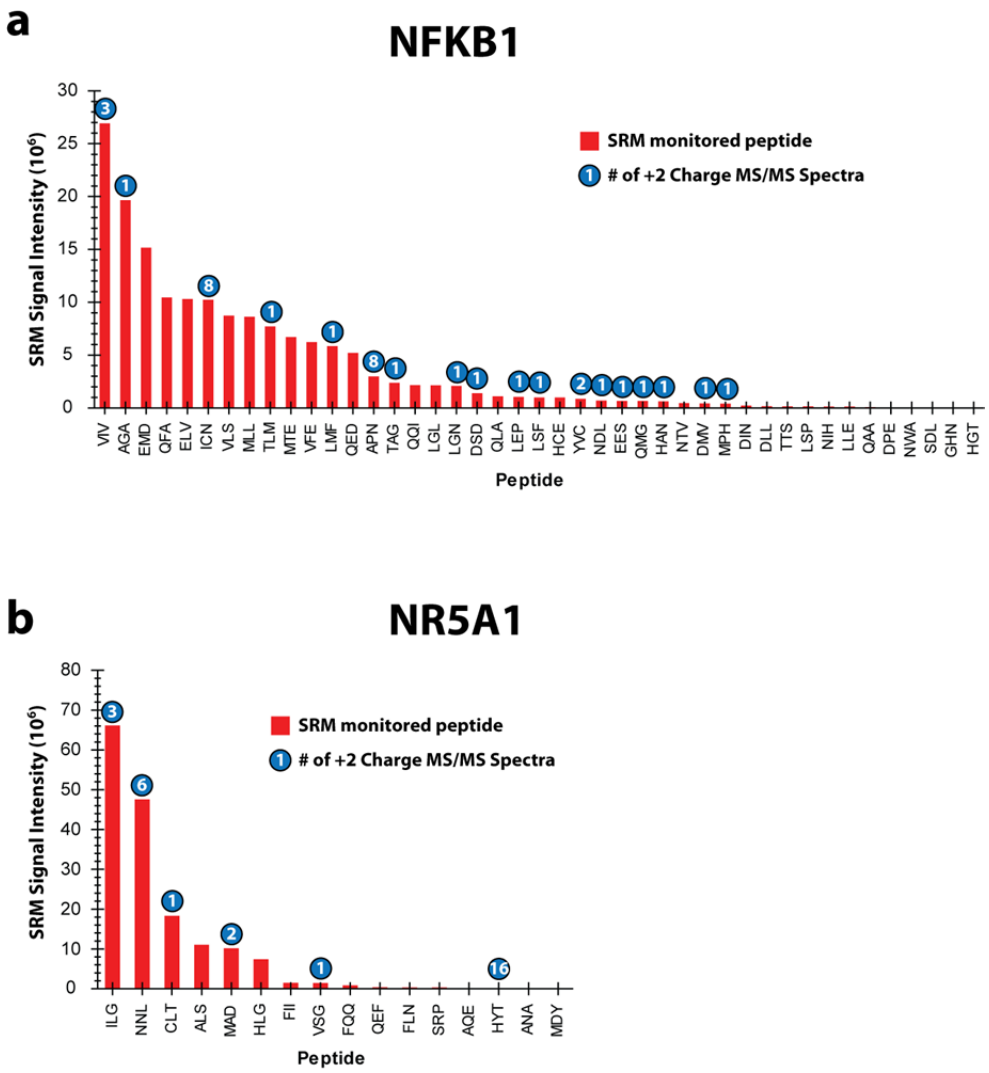


Supplementary Figure 4. Histogram of dot-products for quality score 1 and 2 peptides
Dot-products were calculated using Skyline and the 2011_05_26 NIST release of the H. sapiens Ion Trap peptide spectral library.



Supplementary Figure 5. Spearman correlation of our empirical peptide ranking with ESPPredictor rankings

(a) Box-and-whisker plot showing the distribution of spearman correlations for the 75 proteins with both ESPPredictor scores and empirical SRM peptide signal intensities. The spearman correlation of these 2 rankings for each protein ranged from -0.45 to 0.85 with an average correlation of 0.47. **(b)** A representative comparison of our empirical signal intensity rank for the STAT5A peptides and the ESPPredictor score rank for the same peptides. This comparison shows a better than average correlation (rho = 0.55 versus an average of rho = 0.47).



Supplementary Figure 6. Peptide MS/MS spectrum counts are a poor predictor of targeted peptide signal intensity using selected reaction monitoring-mass spectrometry
(a-b) The NFKB1 **(a)** and NR5A1 **(b)** samples were subjected to 'shotgun' analysis using data-dependent acquisition. For each peptide, the number of +2 charge state spectra identified in this 'shotgun' run is indicated above the SRM signal intensity of that peptide.

Item	Manufacturer	Product #	Cost per unit	Units used per 96 reactions	Cost per 96 reactions
Human In Vitro Protein Expression Kit - DNA (50 reactions)	Pierce	88855	$515	2	$1,030.00
Glutathione Sepharose 4B (10mL)	GE	17-0756-01	$242	0.2	$48.40
PPS Silent Surfactant (5x1 mg vials)	Protein Discovery	21011	$199	1	$199.00
Oasis® MCX plate 30mg/60 µm 1/pkg	Waters	186000250	$326	1	$326.00
Sequencing Grade Modified Trypsin (100µg)	Promega	V5111	$80	0.4	$32.00
Lab reagents	Various	Various	$150	1	$150.00
				Total:	**$1,785.40**
				Cost per reaction:	**$18.60**

Supplementary Figure 7. Reagent cost for generating *in vitro*-synthesized proteins from plasmids

The reagent usage is based on our current working protocol (Methods). The prices used in this calculation are based on manufacture posted prices on May 24th 2011.

SUPPLEMENTARY DATA LEGENDS

Supplementary Data 1. Rank order of proteotypic peptides and fragment ions for targeted proteins

(Pages 9-10) Proteins targeted in this dataset. Columns: Gene Symbol, Gene Description, DNA-binding domain family, mRNA Accession, DNASU Plasmid Repository Clone ID, Page number for targeted data.

(Pages 11-35) For each protein monitored in this dataset, the summed peak intensity for each peptide, as well as the relative contribution of each fragment ion to this signal is displayed. Quality score 1 peptides are labeled in green, quality score 2 peptides are labeled in yellow, quality score 3 peptides are labeled in purple, and quality score 4 peptides are labeled in red.

Proteins Targeted

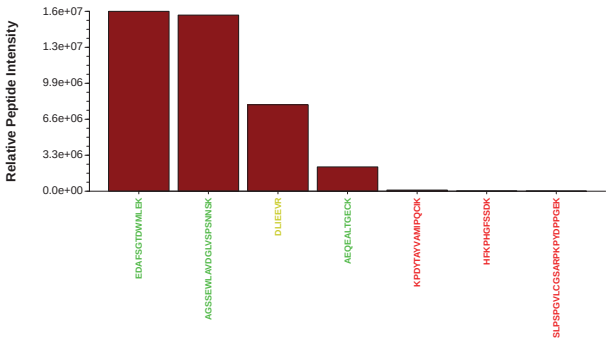
Gene Symbol	Description	DNA-binding domain family	Accession	DNASU Clone ID	Page
ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)	basic-leucine zipper (bZIP)	CR456384	HsCD00301229	pg. 12
ATOH7	atonal homolog 7 (Drosophila)	basic helix-loop-helix (bHLH)	BC032621	HsCD00077283	pg. 12
BATF	basic leucine zipper transcription factor, ATF-like	basic-leucine zipper (bZIP)	NM_006399	HsCD00301458	pg. 12
BHLHB2	basic helix-loop-helix domain containing, class B, 2	basic helix-loop-helix (bHLH)	BC082238	HsCD00299716	pg. 12
CCDC16	coiled-coil domain containing 16	zinc finger, C2H2-type (C2H2)	BC011584	HsCD00078107	pg. 13
CEBPG	CCAAT/enhancer binding protein (C/EBP), gamma	basic-leucine zipper (bZIP)	BC007582	HsCD00077759	pg. 13
CLOCK	clock homolog (mouse)	basic helix-loop-helix (bHLH)	BC041878	HsCD00299832	pg. 13
CREB1	cAMP responsive element binding protein 1	basic-leucine zipper (bZIP)	BC010636	HsCD00077213	pg. 13
CTCF	CCCTC-binding factor (zinc finger protein)	zinc finger, C2H2-type (C2H2)	BC014267	HsCD00078657	pg. 14
DMRT1	doublesex and mab-3 related transcription factor 1	DM DNA-binding domain (DM)	BC040847	HsCD00300076	pg. 14
DMRTC2	DMRT-like family C2	DM DNA-binding domain (DM)	BC029202	HsCD00299763	pg. 14
E2F5	E2F transcription factor 5, p130-binding	TF E2F/dimerisation partner (TDP)	NM_001951	HsCD00302966	pg. 14
ERG	v-ets erythroblastosis virus E26 oncogene like (avian)	ETS-domain (ETS)	BC040168	HsCD00299791	pg. 15
ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	ETS-domain (ETS)	NM_005239	HsCD00301527	pg. 15
ETV3	ets variant gene 3	ETS-domain (ETS)	BC022868	HsCD00077713	pg. 15
EWSR1	Ewing sarcoma breakpoint region 1	ETS-domain (ETS)	CR456490	HsCD00301321	pg. 15
EZH2	enhancer of zeste homolog 2 (Drosophila)	SANT-domain (SANT)	BC010858	HsCD00077678	pg. 16
FEV	FEV (ETS oncogene family)	ETS-domain (ETS)	BC023511	HsCD00079089	pg. 16
FOSB	FBJ murine osteosarcoma viral oncogene homolog B	basic-leucine zipper (bZIP)	NM_006732	HsCD00301650	pg. 16
FOSL2	FOS-like antigen 2	basic-leucine zipper (bZIP)	BC022791	HsCD00079102	pg. 16
FOXD4L1	forkhead box D4 like 1	Forkhead-domain (Forkhead)	NM_012184	HsCD00302896	pg. 17
FOXD4L2	FOXD4-like 2	Forkhead-domain (Forkhead)	BC103887	HsCD00299718	pg. 17
FOXN1	forkhead box N1	Forkhead-domain (Forkhead)	NM_003593	HsCD00302766	pg. 17
FOXR2	forkhead box R2	Forkhead-domain (Forkhead)	BC012934	HsCD00301098	pg. 17
GATA2	GATA binding protein 2	zinc finger, GATA-type (ZNF-GATA)	BC002557	HsCD00077154	pg. 18
GATA3	GATA binding protein 3	zinc finger, GATA-type (ZNF-GATA)	BC006793.1	HsCD00305430	pg. 18
GFI1	growth factor independent 1	zinc finger, C2H2-type (C2H2)	BC032751	HsCD00079248	pg. 18
GMEB1	glucocorticoid modulatory element binding protein 1	SAND-domain (SAND)	BC001473	HsCD00299674	pg. 18
GMEB2	glucocorticoid modulatory element binding protein 2	SAND-domain (SAND)	BC036305	HsCD00299890	pg. 19
GTF2H4	general transcription factor IIH, polypeptide 4, 52kDa	Other	BC004935	HsCD00079304	pg. 19
H1F0	H1 histone family, member 0	Other	CR456502	HsCD00301329	pg. 19
HMGA1	high mobility group AT-hook 1	high mobility group-type (HMG)	NM_002131	HsCD00301489	pg. 19
HMGB2	high-mobility group box 2	high mobility group-type (HMG)	BC001063	HsCD00078150	pg. 20
HOXA10	homeobox A10	Homeodomain (Homeodomain)	BC013971	HsCD00078539	pg. 20
HOXA5	homeobox A5	Homeodomain (Homeodomain)	NM_019102	HsCD00304202	pg. 20
HSF4	heat shock transcription factor 4	Heat-shock factor (Heat Shock)	NM_001538	HsCD00302758	pg. 20
HSFY2	heat shock transcription factor, Y linked 2	Heat-shock factor (Heat Shock)	NM_153716	HsCD00302426	pg. 21
ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	helix-loop-helix (HLH)	D13891	HsCD00301602	pg. 21
ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	helix-loop-helix (HLH)	BC003107	HsCD00078304	pg. 21
IRF1	interferon regulatory factor 1	Interferon regulatory factor (IRF)	BC009483	HsCD00077440	pg. 21
IRF3	interferon regulatory factor 3	Interferon regulatory factor (IRF)	BC009395	HsCD00079105	pg. 22
IRF4	interferon regulatory factor 4	Interferon regulatory factor (IRF)	BC015752	HsCD00077553	pg. 22
IRF5	interferon regulatory factor 5	Interferon regulatory factor (IRF)	BC004139	HsCD00078081	pg. 22
LEF1	lymphoid enhancer-binding factor 1	high mobility group-type (HMG)	BC050632	HsCD00299770	pg. 22
LHX4	LIM homeobox 4	Homeodomain (Homeodomain)	BC011759	HsCD00077458	pg. 23
LIN28	lin-28 homolog (C. elegans)	zinc finger, C2HC-type (C2HC)	BC028566	HsCD00079059	pg. 23
MAD	MAX dimerization protein 1	basic helix-loop-helix leucine zipper (bHLHZ)	L06895	HsCD00301580	pg. 23
MAX	MYC associated factor X	basic helix-loop-helix leucine zipper (bHLHZ)	NM_002382	HsCD00301600	pg. 23
MEF2A	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)	MADs-box (MADs-box)	BC013437	HsCD00299868	pg. 24
MEF2C	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)	MADs-box (MADs-box)	BC026341	HsCD00300012	pg. 24
MYB	v-myb myeloblastosis viral oncogene homolog (avian)	SANT-domain (SANT)	AF104863	HsCD00301531	pg. 24
MYBL2	v-myb myeloblastosis viral oncogene homolog (avian)-like 2	SANT-domain (SANT)	BC007585	HsCD00077770	pg. 24
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	basic helix-loop-helix leucine zipper (bHLHZ)	BC000141	HsCD00076570	pg. 25
MYF6	myogenic factor 6 (herculin)	basic helix-loop-helix (bHLH)	BC017834	HsCD00077638	pg. 25
NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	Cell surface receptor (IPT/TIG)	BC001050	HsCD00077145	pg. 25
NFE2	nuclear factor (erythroid-derived 2), 45kDa	basic-leucine zipper (bZIP)	BC005044	HsCD00079103	pg. 25
NFIA	nuclear factor I/A	nuclear factor I-domain (NFI)	BC022264	HsCD00077446	pg. 26
NFIC	nuclear factor I/C (CCAAT-binding transcription factor)	nuclear factor I-domain (NFI)	BC012120	HsCD00078641	pg. 26
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	Cell surface receptor (IPT/TIG)	BC051765	HsCD00301116	pg. 26
NR1I3	nuclear receptor subfamily 1, group I, member 3	Nuclear hormone receptor (NHR)	BC069626.1	HsCD00304068	pg. 27
NR2E1	nuclear receptor subfamily 2, group E, member 1	Nuclear hormone receptor (NHR)	NM_003269	HsCD00305417	pg. 27
NR3C1	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	Nuclear hormone receptor (NHR)	BC015610	HsCD00300083	pg. 27
NR5A1	nuclear receptor subfamily 5, group A, member 1	Nuclear hormone receptor (NHR)	BC032501	HsCD00299843	pg. 27
OLIG1	oligodendrocyte transcription factor 1	basic helix-loop-helix (bHLH)	BC033290	HsCD00076967	pg. 28
OTX1	orthodenticle homolog 1 (Drosophila)	Homeodomain (Homeodomain)	BC007621	HsCD00077740	pg. 28
PATZ1	POZ (BTB) and AT hook containing zinc finger 1	zinc finger, C2H2-type (C2H2)	CR456613	HsCD00301150	pg. 28
PITX3	paired-like homeodomain transcription factor 3	Homeodomain (Homeodomain)	BC011642	HsCD00078535	pg. 28
PLEK2	pleckstrin 2	Other	BC001226	HsCD00077265	pg. 29
POLR2F	polymerase (RNA) II (DNA directed) polypeptide F	Other	CR456546	HsCD00301192	pg. 29
POU1F1	POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1)	POU-domain (POU)	NM_000306	HsCD00302903	pg. 29
POU3F4	POU domain, class 3, transcription factor 4	POU-domain (POU)	NM_000307	HsCD00303140	pg. 29
POU4F1	POU domain, class 4, transcription factor 1	POU-domain (POU)	NM_006237	HsCD00302919	pg. 30
POU4F3	POU class 4 homeobox 3	POU-domain (POU)	BC104923	HsCD00300001	pg. 30
PPARA	peroxisome proliferator-activated receptor alpha	Nuclear hormone receptor (NHR)	CR456547	HsCD00304173	pg. 30
SF3A2	splicing factor 3a, subunit 2, 66kDa	zinc finger, C2H2-type (C2H2)	BC004434	HsCD00079081	pg. 30

Gene Symbol	Description		Accession	DNASU Clone ID	Page
SOX10	SRY (sex determining region Y)-box 10	high mobility group-type (HMG)	CR456584	HsCD00301364	pg. 31
SOX5	SRY (sex determining region Y)-box 5	high mobility group-type (HMG)	NM_006940	HsCD00301463	pg. 31
SREBF2	sterol regulatory element binding transcription factor 2	basic helix-loop-helix leucine zipper (bHLHZ)	CT841522	HsCD00301198	pg. 31
STAT5A	signal transducer and activator of transcription 5A	Signal Transducers and Activators of Transcription subdomain (STAT)	BC027036	HsCD00076786	pg. 32
STAT5B	signal transducer and activator of transcription 5B	Signal Transducers and Activators of Transcription subdomain (STAT)	BC065227	HsCD00299823	pg. 32
TAF11	TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28kDa	Other	NM_005643	HsCD00301672	pg. 32
TEF	thyrotrophic embryonic factor	basic-leucine zipper (bZIP)	NM_003216	HsCD00304220	pg. 33
TFAP2A	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	Transcription factor AP-2 (AP2)	BC017754	HsCD00077076	pg. 33
TFAP2B	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	Transcription factor AP-2 (AP2)	BC037225	HsCD00076757	pg. 33
TFCP2L1	transcription factor CP2-like 1	CP2 transcription factor (CP2)	BC064698	HsCD00299737	pg. 33
TFCP2L4	transcription factor CP2-like 4	CP2 transcription factor (CP2)	BC036890	HsCD00299801	pg. 34
TFDP2	transcription factor Dp-2 (E2F dimerization partner 2)	TF E2F/dimerisation partner (TDP)	BC021113	HsCD00299708	pg. 34
TGIF2	TGFB-induced factor 2 (TALE family homeobox)	Homeodomain (Homeodomain)	BC012816	HsCD00079074	pg. 34
TP53	tumor protein p53 (Li-Fraumeni syndrome)	p53-like DNA-binding (p53)	BC003596	HsCD00078349	pg. 34
TP73L	tumor protein p73-like	p53-like DNA-binding (p53)	BC039815	HsCD00299815	pg. 35
XBP1	X-box binding protein 1	basic-leucine zipper (bZIP)	CR456611	HsCD00301204	pg. 35
ZMAT5	zinc finger, matrin type 5	zinc finger, C2H2-type (C2H2)	CR456353	HsCD00301319	pg. 35
ZNF496	zinc finger protein 496	zinc finger, C2H2-type (C2H2)	BC007263	HsCD00077733	pg. 35
ZNF530	zinc finger protein 530	zinc finger, C2H2-type (C2H2)	BC060865	HsCD00301131	pg. 36
ZNF70	zinc finger protein 70 (Cos17)	zinc finger, C2H2-type (C2H2)	CR456615	HsCD00301373	pg. 36
ZNF74	zinc finger protein 74	zinc finger, C2H2-type (C2H2)	CR456616	HsCD00301379	pg. 36

Table Legend

Peptide Quality Score
1
2
3
4

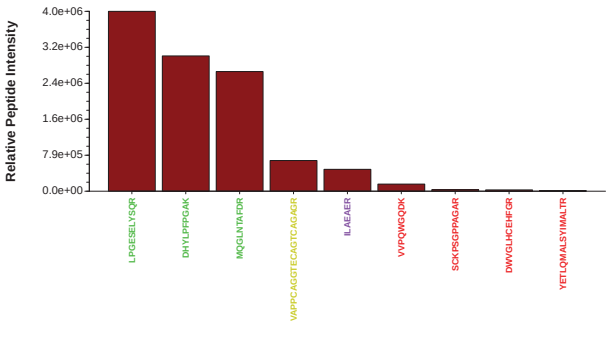
ATF4



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AEQEALTGECK	2	19	27	16	17	13	6									
AGSSEWLAVDGLVSPSNNSK	2	2	2	23	28	8	2	8	10	6	7	2				
EDAFSGTDWMLEK	9	8	8	6	5	22	33	7	2							
DLIEEVR	10	41	49													

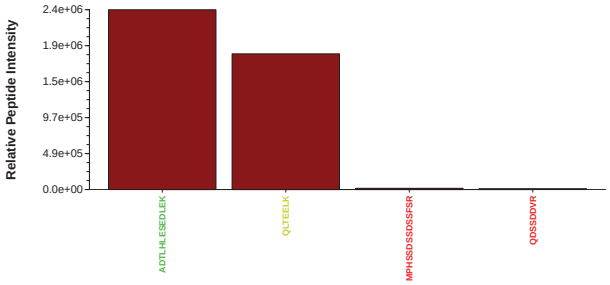
ATOH7



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
DHYLPFPGAK	2	35	2	29	14	17										
LPGESELYSQR	10	14	13	7	21	6	30									
MQGLNTAFDR	6	8	8	18	5	56										
VAPPCAGGTECAGTCAGAGR	2	11	6	7	3	11	8	11	6	4	7	16	8			
ILAEAER	9	9	82													

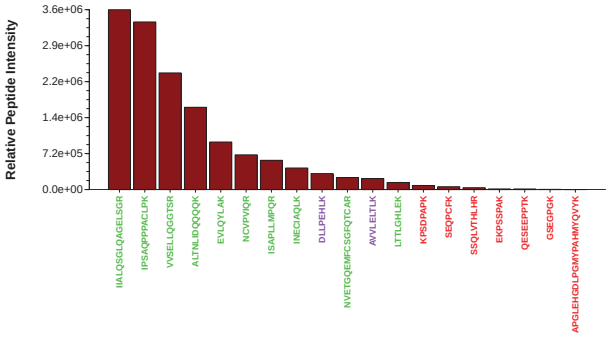
BATF



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ADTLHLESEDLEK	12	8	4	12	19	26	18	1	0							
QLTEELK	18	25	58													

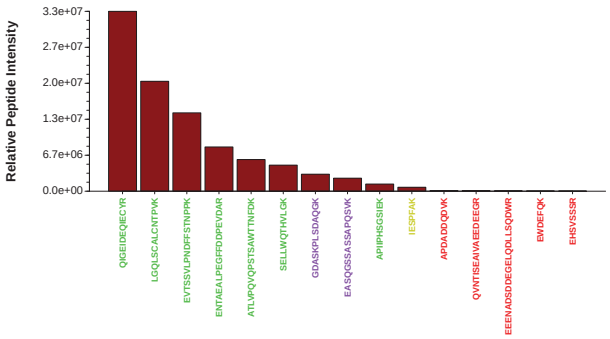
BHLHB2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ALTNLIDQQQQK	4	5	9	34	31	7	5	4								
EVLQYLAK	9	41	23	27												
IIALQSGLQAGELSGR	8	7	2	11	11	9	3	12	21	9	3	4				
INECIAQLK	6	28	14	20	32											
IPSAQPPPACLPK	2	2	0	4	12	72	1	2	4							
ISAPLLMPQR	17	20	22	7	27	6										
LTTLGHLEK	7	4	24	18	48											
NCVPVIQR	2	1	80	16												
NVETGQEMFCSGFQTCAR	7	16	11	4	10	13	16	15	7	2						
VVSELLQGGTSR	1	3	25	20	17	8	3	23								
AVVLELTLK	3	5	17	27	49											
DLLPEHLK	7	1	75	16												

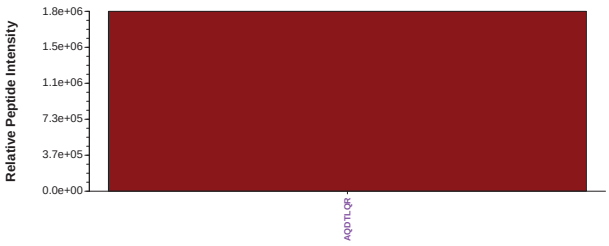
CCDC16



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
APIIPHSGSIEK	0	1	3	7	5	71	8	4								
ATLVPQVQPSTSAWTTNFDK	5	7	8	13	8	4	5	2	2	42	4					
ENTAEALPEGFFDDPEVDAR	6	4	0	44	7	3	5	2	5	1	24					
EVTSSVLPNDFFSTNPPK	22	4	2	5	2	3	1	1	47	12	1					
LGQLSCALCNTPVK	16	6	4	13	9	10	12	23	7	0						
QIGEIDEQIECYR	10	20	11	8	11	21	9	1	10							
SELLWQTHVLGK	15	9	8	13	13	31	10	3								
IESPFAK	5	15	80													
EASQGSSASSAPQSVK	5	1	29	8	8	15	8	9	5	11	0	0				
GDASKPLSDAQGK	3	15	3	6	3	66	3	2	1							

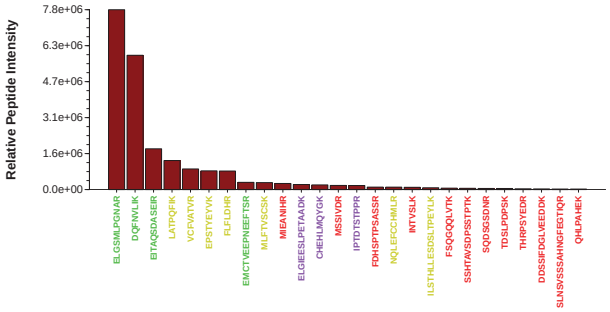
CEBPG



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
AQDTLQR		22	78													

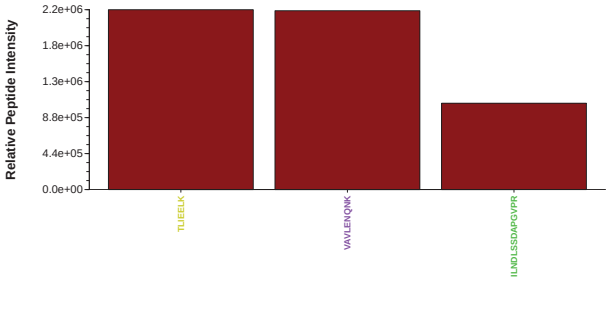
CLOCK



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
DQFNVLIK	13	7	17	63												
EITAQSDASEIR	3	7	12	9	29	16	10	14								
ELGSMLPGNAR	0	2	60	14	7	3	14									
EMCTVEEPNEEFTSR	5	7	4	1	1	42	13	21	4	2						
EPSTYEYVK	12	22	36	10	20											
FLFLDHR	9	19	72													
ILSTHLLESDSLTPEYLK	4	20	34	13	1	4	3	6	8	5						
LATPQFIK	7	4	46	42												
MLFTVSCSK	4	17	12	24	43											
NQLEFCCHMLR	9	8	14	10	22	21	17									
VCFVATVR	4	15	19	62												
CHEHLMQYGK	4	65	12	16	2	1										
ELGIEESLPETAADK	5	5	4	2	41	9	11	11	11	0	2					
IPTDTSTPPR	20	9	9	19	18	26										

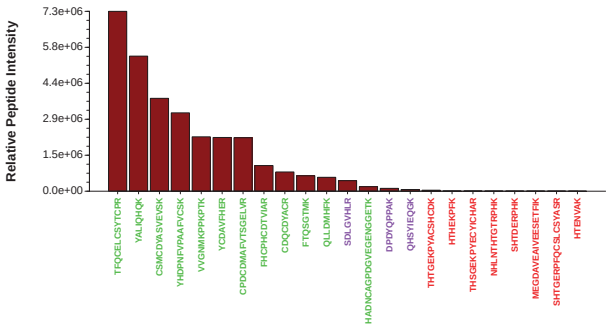
CREB1



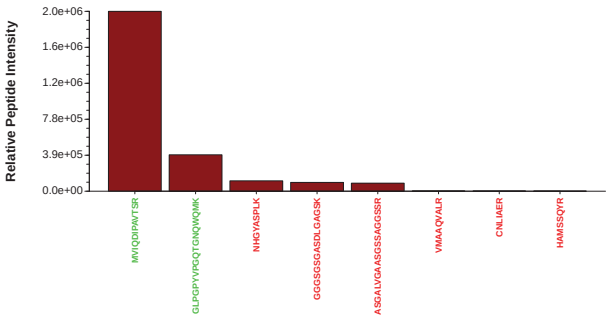
Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
ILNDLSSDAPGVPR	1	3	24	16	5	11	25	5	4	6						
TLIEELK	3	13	85													
VAVLENQNK	2	10	26	35	27											

CTCF



DMRT1



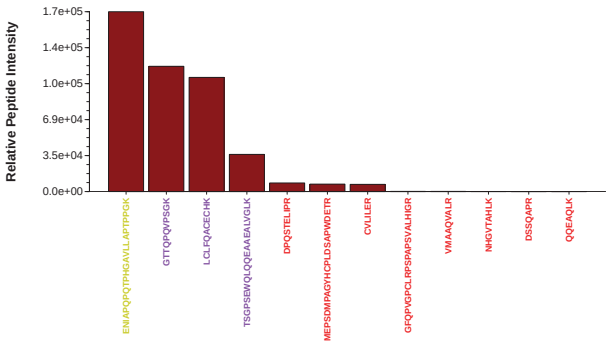
Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
CDQCDYACR	23	23	19	24	11											
CPDCDMAFVTSGELVR	4	1	5	9	22	16	17	13	7	4	1					
CSMCDYASVEVSK	10	15	5	14	14	10	12	13	7							
FHCPHCDTVIAR	3	2	13	4	8	5	52	13								
FTQSGTMK	2	8	33	56												
HADNCAGPDGVEGENGGETK	1	4	14	14	2	15	13	1	20	1	5	9	2			
QLLDMHFK	10	28	29	33												
TFQCELCSYTCPR	11	12	8	17	22	12	7	9	1							
VVGNMKPPKPTK	21	3	3	46	9	5	1	13								
YALIQHQK	15	18	31	36												
YCDAVFHER	9	28	13	17	33											
YHDPNFVPAAFVCSK	10	7	5	3	1	39	6	2	1	21	4					
DPDYQPPAK	4	59	8	12	17											
QHSYIEQGK	13	16	14	10	47											
SDLGVHLR	20	9	56	15												

Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
GLPGPYVPGQTGNQWQMK	3	4	1	1	4	5	1	3	76	2						
MVIQDIPAVTSR	4	1	2	46	9	21	12	6								

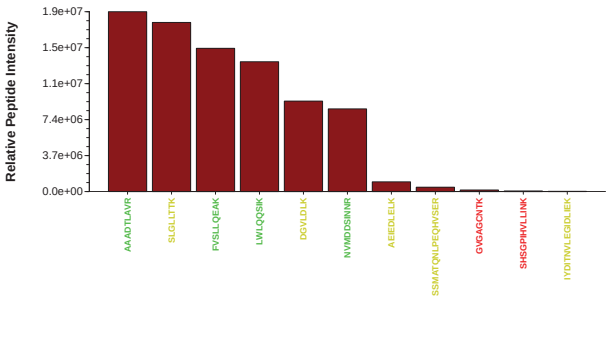
DMRTC2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ENIAPQPQTPHGAVLLAPTPPGK	6	55	1	13	11	2	2	1	0	2	0	5	0			
GTTQPQVPSGK	3	33	8	1	52	1	2									
LCLFQACECHK	9	5		23	12	31	20									
TSGPSEWQLQQEAAEALVGLK	24	13	8	7	3	3	7	7	5	8	11	4				

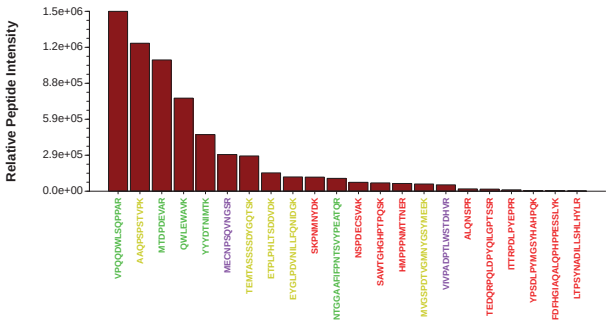
E2F5



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AAADTLAVR	11	10	18	20	41											
FVSLLQEAK	2	5	11	6	77											
LWLQQSIK	4	5	14	77												
NVMDDSINNR	6	3	12	10	20	49										
AEIEDLELK	3	5	14	30	48											
DGVLDLK	12	74	14													
IYDITNVLEGIDLIEK	3	7	14	2	17	12	26	8	4	7	0					
SLGLLTTK	4	7	3	86												
SSMATQNLPEQHVSER	10	4	7	6	1	54	8	8	1	0						

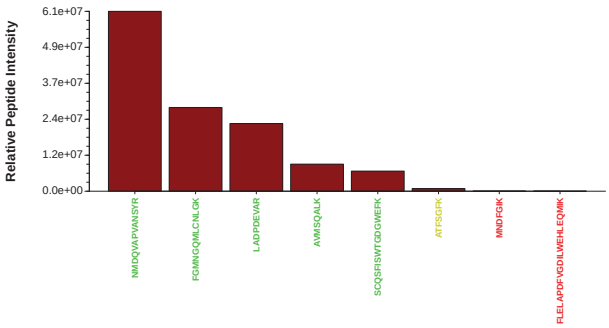
ERG



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
MTDPDEVAR	4	10	5	57	24											
NTGGAAFIFPNTSVYPEATQR	5	5	1	31	15	2	4	1	0	37						
QWLEWAVK	15	30	27	28												
VPQQDWLSQPPAR	2	38	9	16	10	9	7	6	3							
YYYDTNIMTK	9	2	3	11	16	59										
AAQPSPSTVPK	2	2	2	9	4	80	0									
ETPLPHLTSDDVDK	8	9	1	4	16	15	3	42	1	2						
EYGLPDVNILLFQNIDGK	10	2	8	9	26	19	13	3	6	3	0					
MVGSPDTVGMNYGSYMEEK	2	6	11	4	18	12	18	5	21	4						
TEMTASSSSDYGQTSK	9	7	8	10	4	8	12	12	18	8	4	1				
MECNPSQVNGSR	6	16	10	5	5	42	11	5								
VIVPADPTLWSTDHVR	13	2	1	10	20	7	4	29	2	2	10					

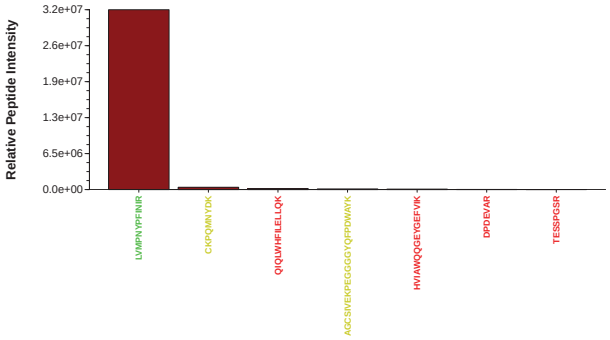
ETS2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AVMSQALK	3	2	20	75												
FGMNGQMLCNLGK	8	12	20	17	18	3	12	8	1							
LADPDEVAR	4	11	7	54	24											
NMDQVAPVANSYR	2	2	4	1	38	34	10	4	5							
SCQSFISWTGDGWEFK	5	3	13	2	13	15	10	27	10	2						
ATFSGFK	7	21	71													

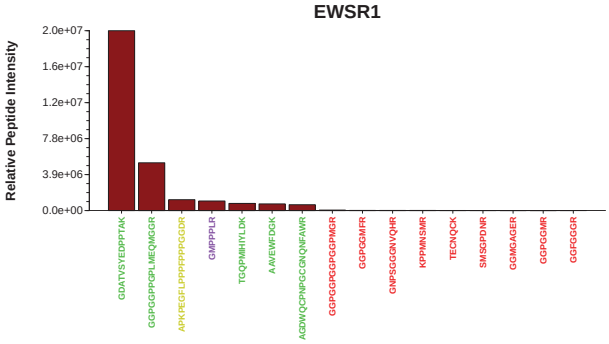
ETV3



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
LVMPNYPFINIR	3	2	3	41	9	6	31	4								
AGCSIVEKPEGGGGYQFPDWAYK	14	34	3	34	8	2	1	1	2	1	1					
CKPQMNYDK	3	4	8	2	84											

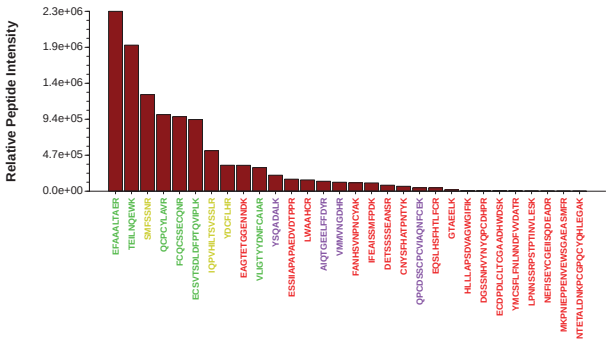
EWSR1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AAVEWFDGK	8	11	34	29	19											
AGDWQCPNPGCGNQNFAWR	15	9	9	6	3	10	5	4	38	2						
GDATVSYEDPPTAK	1	6	56	3	4	6	20	4	1	0						
GGPGGPPGPLMEQMGGR	7	6	6	7	6	1	12	10	25	13	3	2	0			
TGQPMIHIYLDK	5	13	6	15	6	4	51	0								
APKPEGFLPPPPPPPGGDR	1	1	3	3	18	1	3	8	55	5	1	2				
GMPPPLR	12	30	58													

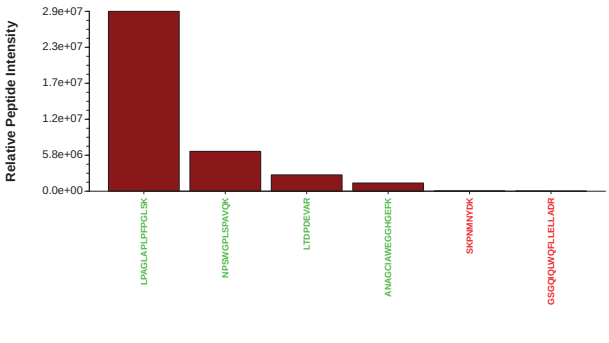
EZH2



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
ECSVTSDLDFPTQVIPLK	51	11	2	1	1	13	6	7	3	2	2					
EFAAALTAER	6	21	23	23	13	14										
FCQCSSECQNR	20	6	2	19	50	2	2									
QCPCYLAVR	10	10	11	7	61											
TEILNQEWK	3	4	24	31	38											
VLIGTYYDNFCAIAR	5	7	10	7	11	17	21	11	3	7						
IQPVHILTSVSSLR	2	3	13	4	10	23	13	11	2	19						
SMFSSNR	8	2	90													
YDCFLHR	26	31	43													
AIQTGEELFFDYR	10	15	22	12	9	3	13	16	1							
QPCDSSCPCVIAQNFCEK	6	11	19	7	31	12	4	1	10							
VMMVNGDHR	2	12	27	28	31											
YSQADALK	15	11	33	41												

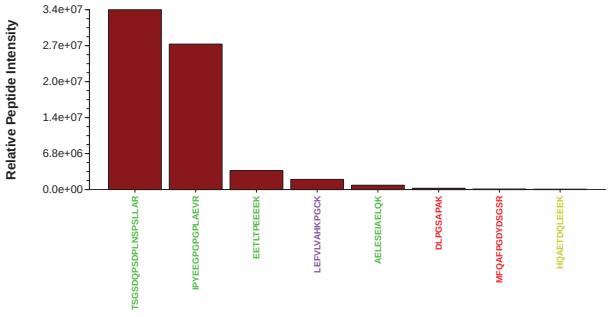
FEV



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
ANAGCIAWEGGHGEFK	1	10	4	3	17	11	18	24	7	2	1					
LPAGLAPLPFPGLSK	1	1	23	1	14	1	36	14	1	3	3					
LTDPDEVAR	4	10	6	54	25											
NPSWGPLSPAVQK	3	1	22	8	1	18	33	10	4							

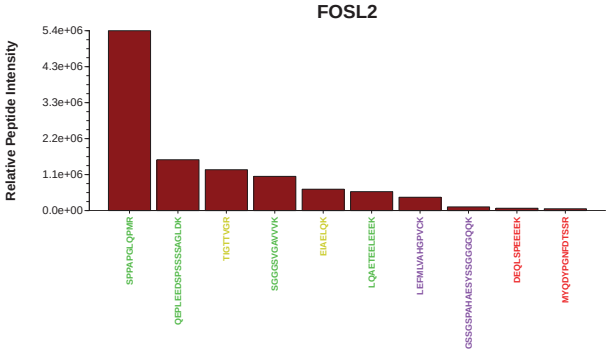
FOSB



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
AELESEIAELQK	9	11	19	12	7	24	11	7								
EETLTPEEEEK	1	4	2	58	23	5	7									
IPYEEGPGPGPLAEVR	3	4	1	5	2	12	4	25	25	10	5	4				
TSGSDQPSDPLNSPSLLAR	5	2	3	14	11	8	1	35	3	2	17	0				
HQAETDQLEEEK	8	8	8	13	5	7	11	40								
LEFVLVAHKPGCK	2	41	9	9	15	11	9	2	1							

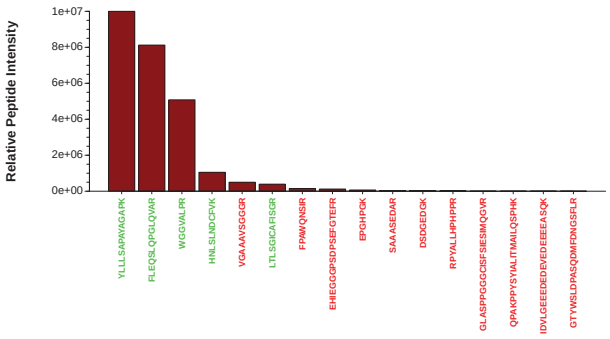
FOSL2



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
LQAETEELEEEK	4	7	6	6	6	14	11	47								
QEPLEEDSPSSSSAGLDK	2	12	6	6	5	3	3	24	16	10	9	4				
SGGGSVGAVVVK	5	4	3	48	17	8	6	9								
SPPAPGLQPMR	5	2	1	3	37	6	45									
EIAELQK	29	22	49													
TIGTTVGR	2	5	4	90												
GSSGSPAHAESYSSGGGGQQK	2	7	7	9	12	13	12		15	7	12	4	1			
LEFMLVAHGPVCK	1	11	25	16	17	14	9	4	3							

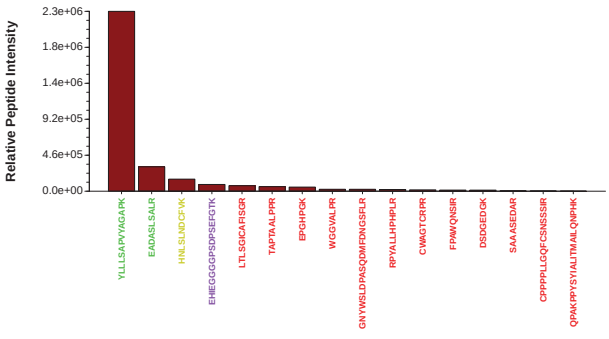
FOXD4L1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
FLEQSLQPGLQVAR	3	4	2	2	58	14	6	8	2	1						
HNLSLNDCFVK	3	20	5	8	7	26	31									
LTLSGICAFISGR	10	7	7	7	25	7	14	17	5							
WGGVALPR	9	51	11	29												
YLLLSAPAYAGAPK	1	3	2	1	1	30	11	29	14	8						

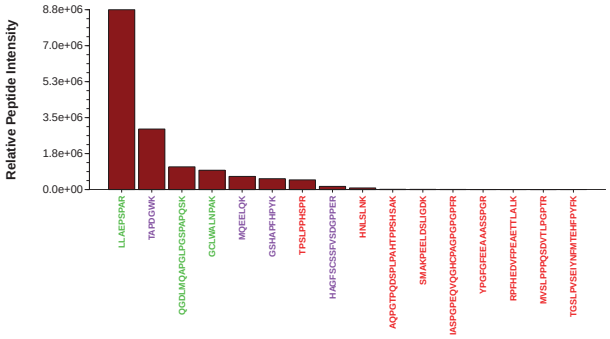
FOXD4L2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
EADASLSALR	8	31	18	26	7	11										
YLLLSAPVYAGAPK	1	4	3	3	1	27	11	28	14	8						
HNLSLNDCFVK	6	20	5	7	5	23	34									
EHIEGGGGPSDPSEFGTK	8	12	4	1	46	3	1	6	3	5	1	6	3			

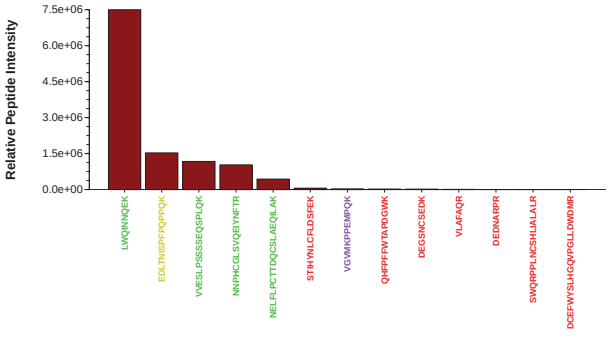
FOXN1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
GCLWALNPAK	14	10	12	22	29	13										
LLAEPSPAR	2	1	51	8	39											
QGDLMQAPGLPGSPAPQSK	0	13	1	8	1	1	32	1	2	34	7	1	0			
GSHAPFHPYK	24	10	2	45	18	1										
HAGFSCSSFVSDGPPER	1	3	74	1	6	3	3	4	3	2	1					
MQEELQK	8	11	80													
TAPDGWK	8	6	86													

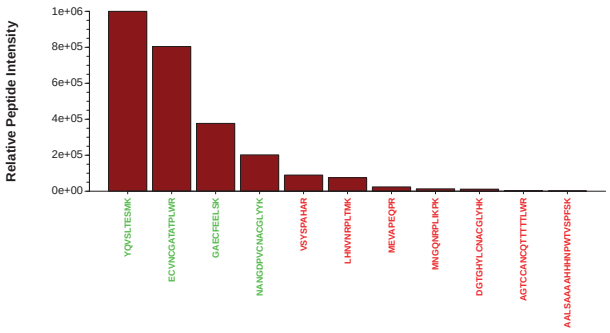
FOXR2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
LWQINNQEK	1	5	19	30	44											
NELFLPCTTDQCSLAEQILAK	18	12	23	8	12	4	6	6	5	4	2					
NNPHCGLSVQEIYNFTR	4	12	16	9	7	13	7	21	2	10						
VVESLPSSSSEQSPLQK	1	15	8	2	2	3	4	3	2	58	1	1				
EDLTNISPFPQPPQK	3	30	1	8	2	21	29	3	3	1	0					
VGVMKPPEMPQK	6	9	0	5	64	6	6	3								

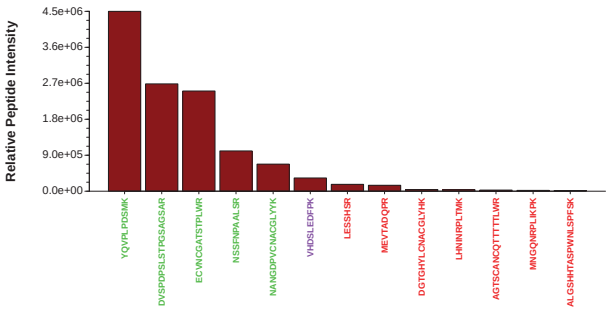
GATA2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ECVNCGATATPLWR	1	33	11	9	13	4	14	6	7	2						
GAECFEELSK	12	15	19	27	20	7										
NANGDPVCNACGLYYK	20	2	8	10	6	7	16	2	28	1						
YQVSLTESMK	7	4	8	5	29	47										

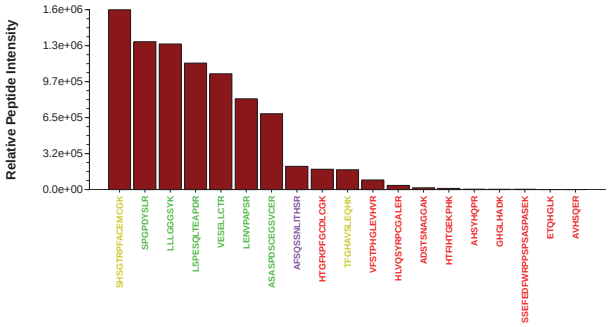
GATA3



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
DVSPDPSLSTPGSAGSAR	1	4	3	1	2	34	9	21	5	3	15	1	2	0		
ECVNCGATSTPLWR	1	31	7	10	15	4	16	7	7	3						
NANGDPVCNACGLYYK	16	3	10	8	7	8	17	2	28	1						
NSSFNPAALSR	4	6	3	42	18	12	14									
YQVPLPDSMK	4	1	20	2	54	19										
VHDSLEDFPK	21	8	3	3	12	53										

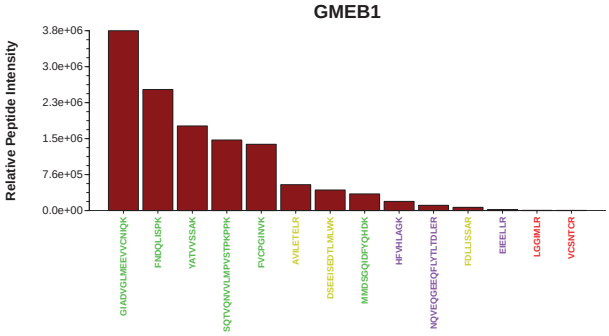
GFI1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ASASPDSCEGSVCER	11	7	5	17	7	6	14	5	24	2	0					
LENVPAPSR	2	2	81	3	12											
LLLGGGSYK	1		4	27	68											
LSPESQLTEAPDR	12	19	8	19	11	4	17	4	5							
SPGPDYSLR	8	22	15	18	38											
VESELLCTR	12	20	15	5	49											
SHSGTRPFACEMCGK	6	2	87	3	0	0	0	0	0	0						
TFGHAVSLEQHK	4	8	6	22	22	30	2	8								
AFSQSSNLITHSR	9	28	15	3	5	11	23	2	5							

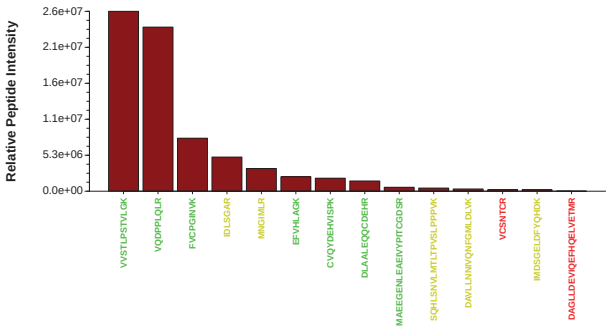
GMEB1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
FNDQLISPK	47	10	9	6	28											
FVCPGINVK	4	3	4	56	32											
GIADVGLMEEVVCNIQK	5	10	20	15	8	8	13	13	2	7						
MMDSGQIDFYQHDK	12	7	11	15	26	15	3	6	4	2						
SQTVQNVVLMPVSTPKPPK	18	1	6	2	3	1	27	12	13	15	2					
YATVVSSAK	3	10	24	13	50											
AVILETELR	2	7	36	31	24											
DSEEISEDTLMLWK	12	12	4	4	8	7	41	9	3	0						
FDLLISSAR	1	17	46	20	16											
EIEELLR	13	41	46													
HFVHLAGK	25	17	15	43												
NQVEQGEEQFLYTLTDLER	8	7	15	11	19	20	10	8	2							

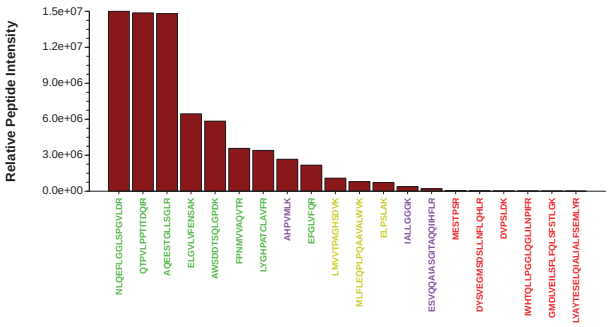
GMEB2



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
CVQYDEHVISPK	32	9	5	6	7	12	17	13								
DLAALEQQCDEHR	10	6	13	11	14	26	12	6	2							
EFVHLAGK	24	27	28	20												
FVCPGINVK	6	6	8	48	33											
MAEEGENLEAEIVYPITCGDSR	0	3	4	7	1	43	23	10	4	2	2					
VQDPPLQLR	2	1	8	72	16											
VVSTLPSTVLGK	4	2	1	1	49	5	4	33								
DAVLLNNIVQNFGMLDLVK	7	17	9	4	13	6	9	9	20	4	2					
IDLSGAR	10	29	61													
IMDSGELDFYQHDK	18	5	10	16	17	12	3	8	6	6						
MNGIMLR	36	8	57													
SQHLSNVLMTLTPVSLPPPVK	3	14	48	3	2	0	8	7	3	5	3	2				

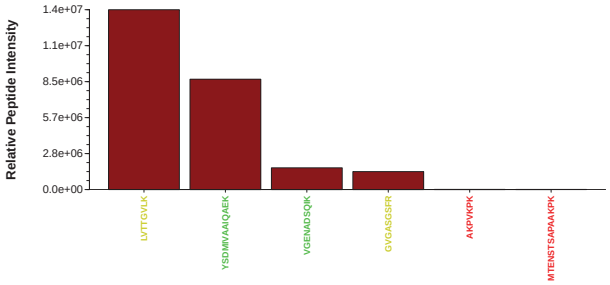
GTF2H4



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
AQEESTGLLSGLR	6	13	11	3	13	9	15	10	19							
AWSDDTSQLGPDK	7	23	8	2	7	6	6	7	34							
EFGLVFQR	28	43	4	25												
ELGVLVFENSAK	2	5	5	25	22	29	2	10								
FPNMVVAQVTR	2	5	17	32	20	9	14									
LYGHPATCLAVFR	2	2	2	2	4	5	80	1	3							
NLQEFLGGLSPGVLDR	5	1	1	21	16	3	8	26	10	5	3	0				
QTPVLPPTITDQIR	5	1	2	1	2	6	69	5	1	8						
ELPSLAK	4	21	75													
LMVVTPAGHSDVK	1	3	2	4	3	43	22	14	9							
MLFLEQPLPQAAVALWVK	2	1	4	2	1	2	1	28	3	54	2					
AHPVMLK	9	3	88													
ESVQQAIASGITAQQIIHFLR	8	26	13	3	6	6	7	15	1	7	9					
IALLGGGK	2	18	21	58												

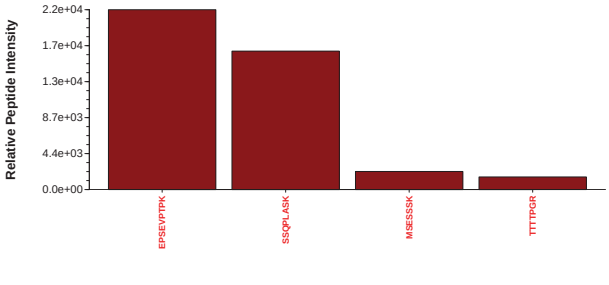
H1F0



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
VGENADSQIK	4	20	27	18	24	8										
YSDMIVAAIQAEK	5	6	7	10	21	35	10	2	3							
GVGASGSFR	2	10	26	5	57											
LVTTGVLK	4	4	5	86												

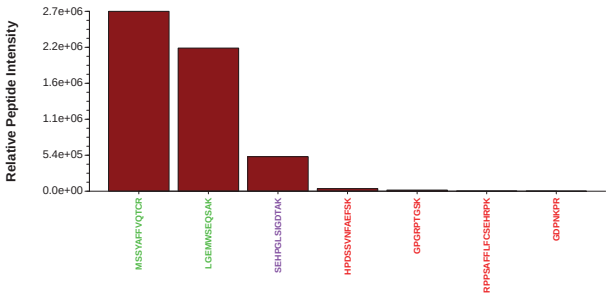
HMGA1



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18

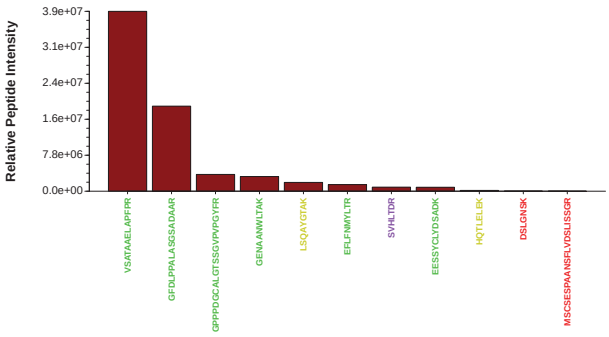
HMGB2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
LGEMWSEQSAK	7	5	7	33	31	14	3									
MSSYAFFVQTCR	7	14	15	21	24	14	3	3								
SEHPGLSIGDTAK	8	1	7	3	6	2	8	64	1							

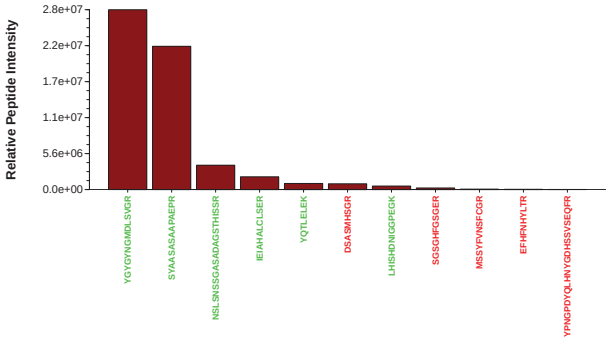
HOXA10



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
EESSYCLYDSADK	3	12	15	19	13	22	7	3	6							
EFLFNMYLTR	9	17	13	26	23	12										
GENAANWLTAK	17	8	9	17	27	10	11									
GFDLPPALASGSADAAR	8	1	1	1	5	10	14	6	4	17	34	0	0			
GPPPDGCALGTSSGVPVPGYFR	1	2	39	1	31	2	4	3	4	2	9	2				
VSATAAELAPFPR	1	26	19	12	11	17	9	4	2							
HQTLELEK	23	9	8	60												
LSQAYGTAK	3	14	20	37	25											
SVHLTDR	24	50	25													

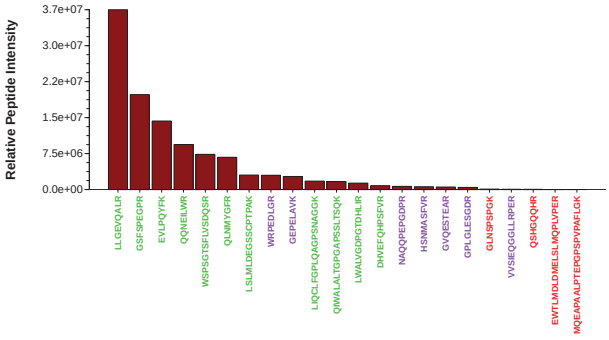
HOXA5



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
IEIAHALCLSER	4	3	9	19	29	20	12	4								
LHISHDNIGGPEGK	1	8	8	18	4	8	7	8	23	16						
NSLSNSSGASADAGSTHISSR	21	8	7	4	2	7	15	6	5	9	2	6	4	2		
SYAASASAAPAEPR	1	1	27	16	10	19	9	11	5	3						
YGYGYNGMDLSVGR	4	13	14	10	4	17	17	6	13	1						
YQTLELEK	8	8	10	74												

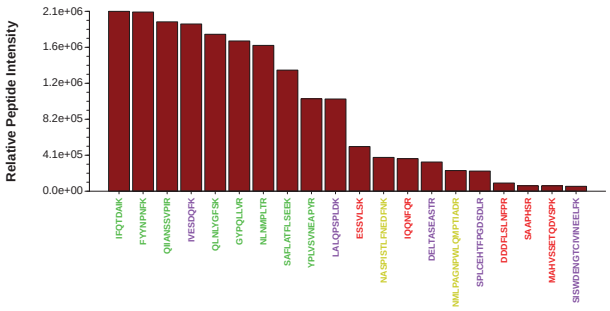
HSF4



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
DHVEFQHPSFVR	1	2	27	16	10	14	20	9								
EVLPQYFK	7	4	73	15												
GSFSPEGPR	6	24	42	22	6											
LIQCLFGPLQAGPSNAGGK	7	6	3	1	7	16	12	3	0	13	24	9	1			
LLGEVQALR	3	9	13	3	71											
LSLMLDEGSSCPTPAK	19	1	11	6	6	5	17	8	17	9	2					
LWALVGDPGTDHLIR	1	12	1	1	1	54	3	17	7	1	1					
QIWALALTGPGAPSSLTSQK	4	6	1	2	1	19	3	1	23	25	11	3	1			
QLNMYGFR	31	32	10	26												
QQNEILWR	43	27	8	21												
WSPSGTSFLVSDQSR	8	4	23	17	12	9	11	3	6	4	3					
GEPELAVK	3	7	5	86												
GPLGLESGDR	3	30	18	9	30	11										
GVQESTEAR	9	11	28	33	19											
HSNMASFVR	3	10	15	17	56											
NAQQPEPGDPR	3	6	2	7	3	23	55									
VVSIEQGGLLRPER	57	13	7	5	5	5	2	3	1	2						
WRPEDLGR	61	6	1	32												

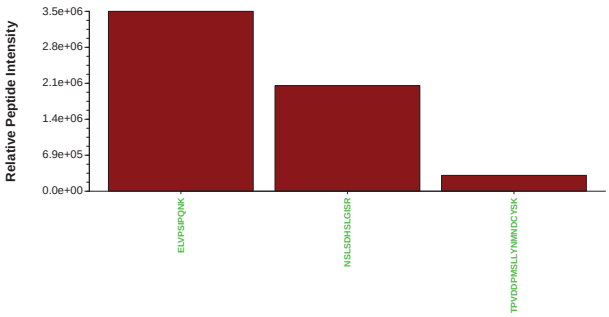
HSFY2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
FYYNPNFK	2	28	14	56												
GYPQLLVR	9	26	15	50												
IFQTDAIK	6	4	24	67												
NLNMPLTR	2	31	16	51												
QIANSSVPIR	21	5	10	10	13	19	22									
QLNLYGFSK	4	32	37	10	17											
SAFLATFLSEEK	3	14	7	10	22	31	11	3								
YPLVSVNEAPYR	7	11	4	11	7	33	17	10								
NASPISTLFNEDFNK	59	7	18	12	2	1	1	0	0	0						
NMLPAGNPWLQMPTIADR	11	3	2	27	13	11	8	2	21	1	1					
DELTASEASTR	3	10	7	19	24	31	7									
IVESDQFK	3	3	15	79												
LALQPSPLDK	3	20	4	61	6	5										
SISWDENGTCIVINEELFK	5	9	7	27	19	17	5	7	2	2						
SPLCEHTFPGDSDLR	16	14	16	30	5	9	10	0	0	0						

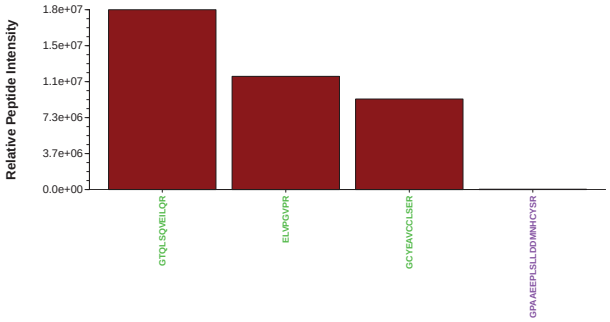
ID2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ELVPSIPQNK	0	35	3	5	53	3										
NSLSDHSLGISR	1	12	8	20	22	9	23	5								
TPVDDPMSLLYNMNDCYSK	8	14	6	9	9	17	20	14	3							

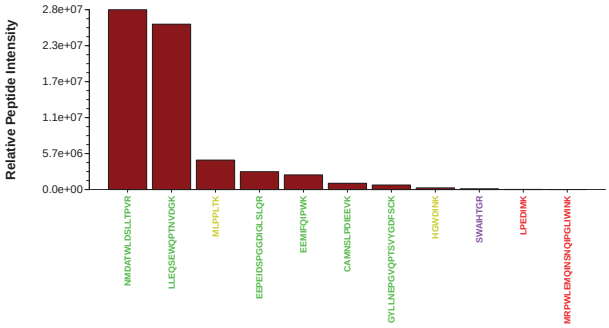
ID3



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ELVPGVPR	1	11	82	6												
GCYEAVCCLSER	11	4	8	28	17	18	9	5								
GTQLSQVEILQR	14	10	16	13	9	28	10	0								
GPAAEEPLSLLDDMNHCYSR	10	15	9	8	18	15	11	10	4							

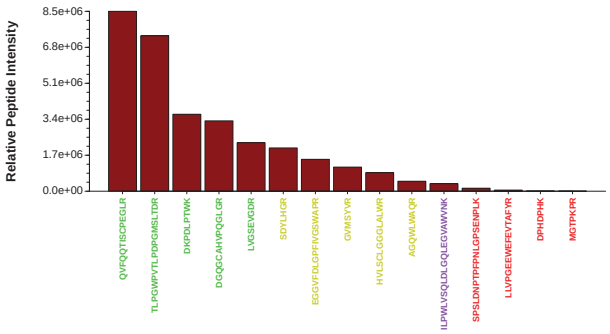
IRF1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
CAMNSLPDIEEVK	3	6	7	2	46	9	11	10	7							
EEMIFQIPWK	36	9	7	35	8	6										
EEPEIDSPGGDIGLSLQR	2	12	2	10	10	2	2	3	30	13	11	2				
GYLLNEPGVQPTSVYGDFSCK	11	12	4	14	18	4	5	1	27	3	1					
LLEQSEWQPTNVDGK	13	6	5	2	53	4	9	3	5	1						
NMDATWLDSLLTPVR	20	20	7	3	8	12	13	9	5	2	2					
HGWDINK	62	12	26													
MLPPLTK	1	8	92													
SWAIHTGR	8	27	16	49												

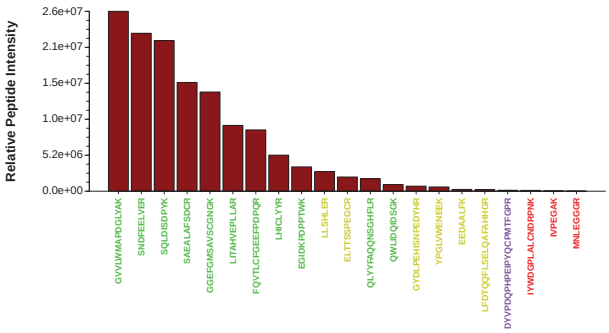
IRF3



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
DGQGCAHVPQGLGR	1	5	2	73		10	5	1	3	0						
DKPDLPTWK	1	15	17	4	64											
LVGSEVGDR	4	8	3	4	81											
QVFQQTISCPEGLR	7	1	18	10	26	10	15	7	3	4						
TLPGWPVTLPDPGMSLTDR	1	1	2	0	0	48	1	23	4	5	1	14				
AGQWLWAQR	16	39	24	21	1											
EGGVFDLGPFIVGSWAPR	8	3	3	17	11	4	1	17	27	6	3					
GVMSYVR	8	35	57													
HVLSCLGGGLALWR	2	4	1	3	4	15	7	6	17	41						
SDYLHGR	49	31	20													
ILPWLVSQLDLGQLEGVAWVNK	12	17	25	2	14	7	5	2	9	4	2					

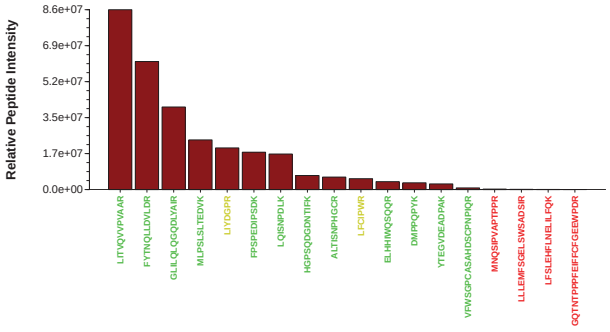
IRF4



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
EGIDKPDPPTWK	2	3	88	0	5	1	1	0								
FQVTLCFGEEFPDPQR	36	2	20	10	4	2	10	7	8	2						
GGEFGMSAVSCGNGK	2	7	6	24	11	10	20	5	12	2	0					
GVVLWMAPDGLYAK	4	1	7	1	25	22	23	14	3	0						
LHICLYYR	3	5	33	59												
LITAHVEPLLAR	1	1	23	21	25	15	5	9								
QLYYFAQQNSGHFLR	8	5	6	7	12	12	13	19	13	5						
QWLIDQIDSGK	14	13	5	8	30	16	13									
SAEALAFSDCR	3	12	27	35	13	8	2									
SNDFEELVER	12	14	19	24	15	16										
SQLDISDPYK	23	4	10	10	21	33										
EEDAALFK	25	32	13	31												
ELTTSSPEGCR	11	2		27	27	15	18									
GYDLPEHISNPEDYHR	17	6	1	21	5	26	11	6	2	6						
LFDTQQPLSELQAFAHHGR	18	14	11	9	9	7	8	3	13	8						
LLSHLER	16	13	71													
YPGLVWENEEK	2	10	10	32	25	4	18									
DYVPDQPHPEIPYQCPMTFGPR	12	7	4	1	17	7	3	1	42	4						

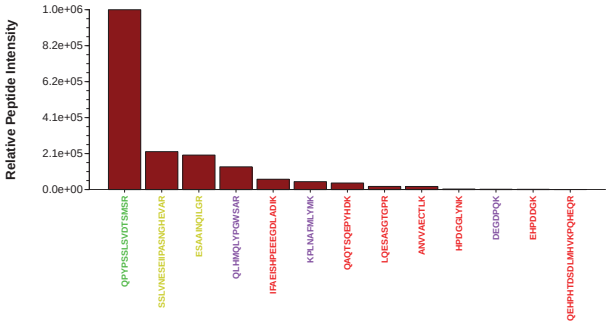
IRF5



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
ALTISNPHGCR	9	4	30	11	29	7	11									
DMPPQPYK	12	1	17	70												
ELHHIWQSQQR	1	9	7	37	23	23	1									
FPSPEDIPSDK	3	35	13	5	4	23	16									
FYTNQLLDVLDR	8	13	17	21	16	5	7	13								
GLILQLQGQDLYAIR	12	9	8	5	3	25	14	16	6	2						
HGPSQDGDNTIFK	5	3	28	1	27	8	2	5	20							
LITVQVVPVAAR	1	1	24	18	17	24	5	9								
LQISNPDLK	1	16	4	21	59											
MLPSLSLTEDVK	3	3	8	6	20	12	13	36								
VFWSGPCASAHDSCPNPIQR	2	17	1	18	5	20	10	12	7	7	2					
YTEGVDEADPAK	29	4	5	5	19	4	16	17								
LFCIPWR	29	15	55													
LIYDGPR	30	6	64													

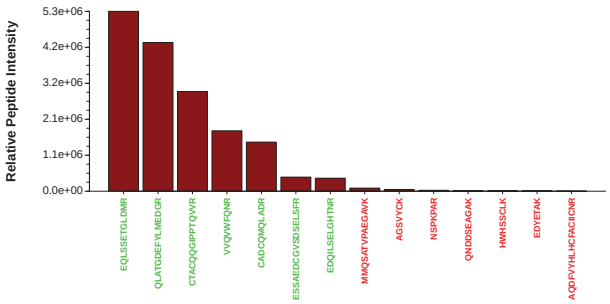
LEF1



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
QPYPSSLSVDTSMSR	2	4	13	13	7	28	9	8	4	9	2					
ESAAINQILGR	15	12		51	13	7	2									
SSLVNESEIIPASNGHEVAR	1	2	2	1	2	3	2	66	14	6	1	1				
DEGDPQK	21	13	66													
KPLNAFMLYMK	11	11	7	8	12	27	24									
QLHMQLYPGWSAR	6	1	2	46	12	9	13	8	3							

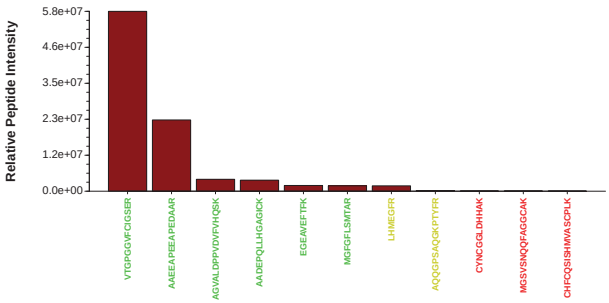
LHX4



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
CADCQMQLADR	18	17	12	17	9	9	18									
CTACQQGIPPTQVVR	1	2	0	2	80	2	8	3	1	1	0					
EDQILSELGHTNR	5	6	16	12	6	26	23	4	0							
EQLSSETGLDMR	7	2	12	16	9	17	24	14								
ESSAEDCGVSDSELSFR	11	9	3	13	5	32	4	12	7	6						
QLATGDEFYLMEDGR	7	12	18	15	14	10	6	4	8	6						
VVQVWFQNR	7	20	35	21	16											

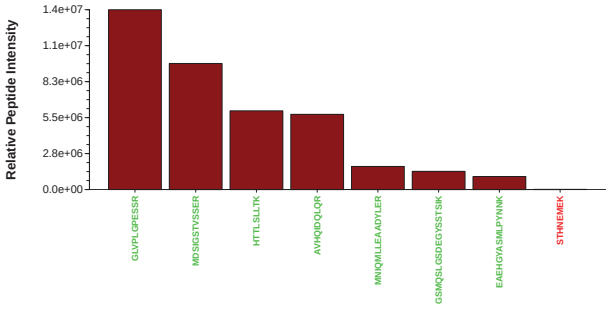
LIN28



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
AADEPQLLHGAGICK	2	9	4	24	23	12	7	2	18	0	0					
AAEEAPEEAPEDAAR	6	2	0	16	10	5	2	53	5	1	0					
AGVALDPPVDVFVHQSK	3	17	4	5	4	3	1	6	51	6	1					
EGEAVEFTFK	8	14	45	18	14	2										
MGFGFLSMTAR	4	7	29	25	9	22	3									
VTGPGGVFCIGSER	3	16	6	11	32	6	8	8	3	6						
AQQGPSAQGKPTYFR	2	2	38	3	15	8	5	3	9	11	3					
LHMEGFR	12	14	73													

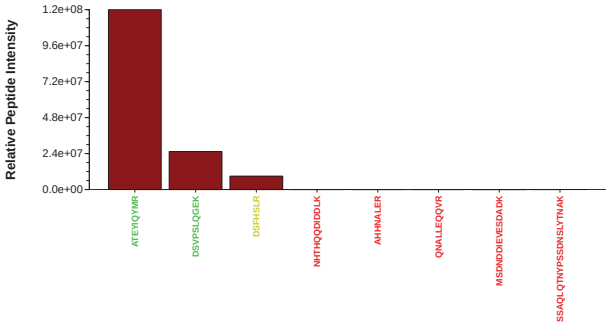
MAD



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
AVHQIDQLQR	3	21	17	26	27	5										
EAEHGYASMLPYNNK	15	3	50	6	3	5	7	3	8	1						
GLVPLGPESSR	3	1	7	30	8	51	1									
GSMQSLGSDEGYSSTSIK	9	5	6	11	2	8	6	5	8	29	6	6				
HTTLSLLTK	14	6	10	10	61											
MDSIGSTVSSER	4	12	6	8	10	47	5	8								
MNIQMLLEAADYLER	1	7	5	9	14	18	20	13	9	3						

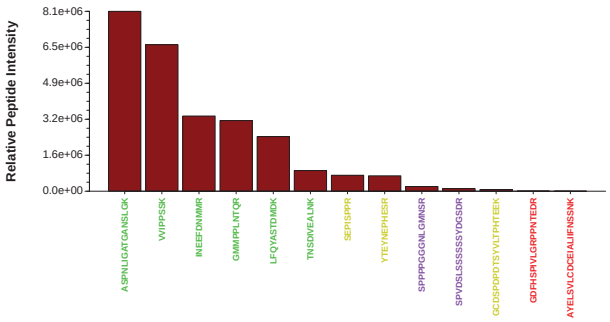
MAX



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
ATEYIQYMR	14	26	22	23	15											
DSVPSLQGEK	3	2	3	8	82	2										
DSFHSLR	42	45	13													

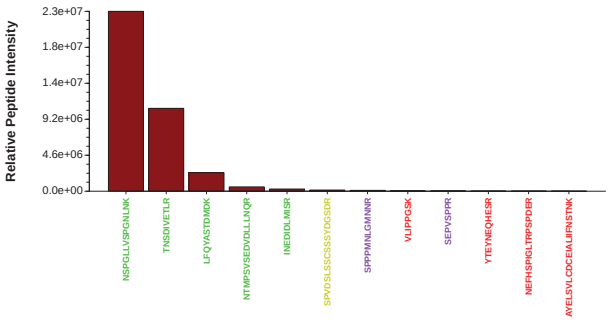
MEF2A



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ASPNLIGATGANSLGK	2	3	3	2	8	9	6	43	18	4	1	0				
GMMPPLNTQR	3	6	6	29	52	4										
INEEFDNMMR	4	15	16	28	16	21										
LFQYASTDMDK	7	5	7	19	22	29	10									
TNSDIVEALNK	6	12	16	27	10	7	22									
VVIPPSSK	1	9	51	39												
GCDSPDPDTSYVLTPHTEEK	3	11	3	46	12	10	7	2	3	3						
SEPISPPR	8	51	11	30												
YTEYNEPHESR	7	3	47	8	14	12	8									
SPPPPGGGNLGMNSR	1	1	4	2	1	2	3	7	28	26	26					
SPVDSLSSSSSSYDGSDR	1	19	5	5	5	5	6	12	15	22	2	4				

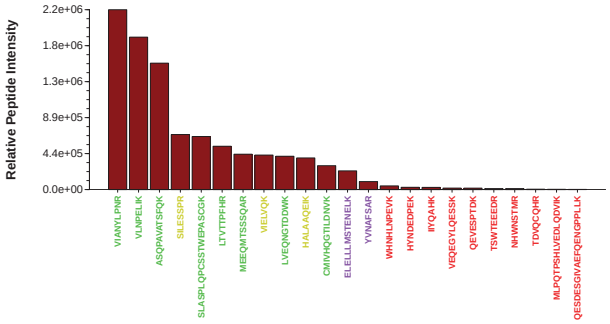
MEF2C



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
INEDIDLMISR	9	12	12	25	11	14	17									
LFQYASTDMDK	5	5	7	18	23	30	12									
NSPGLLVSPGNLNK	1	1	1	21	30	20	14	2	3	6						
NTMPSVSEDVDLLLNQR	13	18	12	9	11	6	7	4	18	2	1					
TNSDIVETLR	6	15	32	14	9	25										
SPVDSLSSCSSSYDGSDR	2	22	8	5	6	6	10	8	14	17	2					
SEPVSPPR	7	41	6	45												
SPPPMNLGMNNR	3	3	8	6	7	4	21	48								

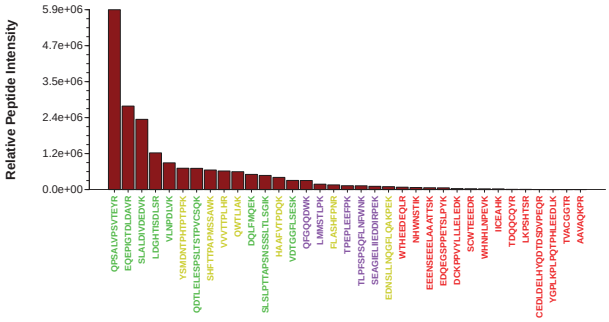
MYB



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ASQPAVATSFQK	2	6	7	19	7	4	54	0								
CMIVHQGTILDNVK	16	10	9	3	2	22	8	18	11	2						
LTVTTPFHR	2	30	15	36	17											
LVEQNGTDDWK	6	8	5	17	20	8	36									
MEEQMTSSSQAR	3	7	9	20	28	19	8	7								
SLASPLQPCSSTWEPASCGK	4	10	2	50	6	3	2	2	2	1	19					
VIANYLPNR	20	13	9	12	46											
VLNPELIK	5	2	32	62												
HALAAQEIK	4	3	9	20	64											
SILESSPR	3	25	21	51												
VIELVQK	5	8	86													
ELELLLMSTENELK	1	9	5	8	21	24	23	8	1	0						
YVNAFSAR	9	11	12	67												

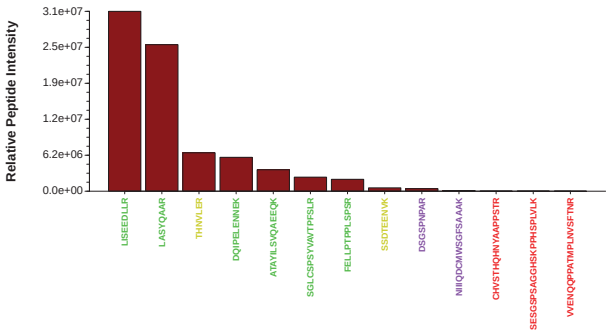
MYBL2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
DQLFMQEK	5	19	36	40												
EQEPIGTDLDAVR	7	3	7	8	5	31	4	31	4							
LDGHTISDLSR	21	3	19	22	31	1	2									
QDTLELESPSLTSTPVCSQK	5	10	1	33	5	7	8	1	1	19	10					
QPSALVPSVTEYR	1	3	1	2	68	13	5	1	7							
SLALDIVDEDVK	3	5	9	17	11	25	12	18								
SLSLPTTAPSNSSSLTLSGIK	5	10	5	6	1	2	2	3	2	2	52	9	3			
VDTGGFLSESK	4	19	11	7	13	19	27									
VLNPDLVK	4	3	29	63												
EDNSLLNQGFLQAKPEK	42	9	5	5	4	2	13	6	10	4	1					
FLASHFPNR	22	24	11	14	29											
HAAFVTPDQK	2	34	14	5	10	35										
QWTLIAK	32	21	47													
SHFTTPAPMSSAWK	2	3	3	0	13	2	40	8	15	14						
VVVTTPLHR	0	19	9	30	42											
YSMDNTPHTPTPFK	21	2	19	10	3	31	4	4	4	1						
LMMSTLPK	1	1	11	87												
QFGQQDWK	24	30	6	41												
SEAGIELIIEDDIRPEK	6	7	19	9	5	12	19	13	7	2						
TLPFSPSQFLNFWNK	12	9	10	6	8	2	3	38	12							
TPEPLEEFPK	10	8	9	7	54	12										

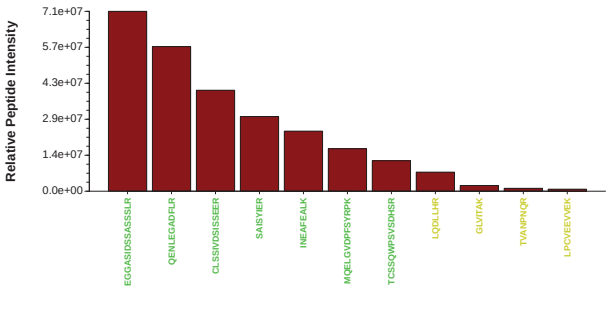
MYC



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
ATAYILSVQAEEQK	5	6	9	10	9	25	26	8	1	0						
DQIPELENNEK	1	4	3	5	2	81	5									
FELLPTPPLSPSR	2	2	1	2	11	2	75	3	2							
LASYQAAR	7	15	10	68												
LISEEDLLR	3	4	7	5	81											
SGLCSPSYVAVTPFSLR	2	0	21	25	7	19	10	3	2	11	1					
SSDTEENVK	5	12	16	22	44											
THNVLER	10	14	76													
DSGSPNPAR	8	3	63	5	21											
NIIQDCMWSGFSAAAK	6	7	14	2	14	18	17	6	8	8	1					

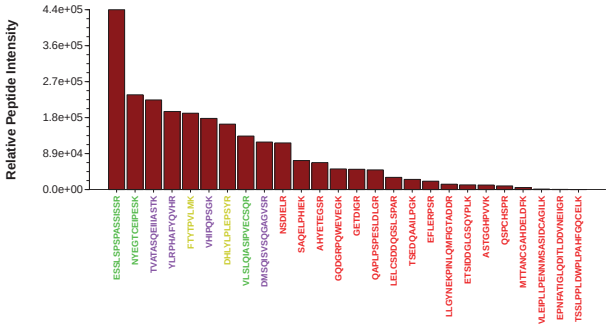
MYF6



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
CLSSIVDSISSEER	1	5	12	4	12	17	27	5	6	12						
EGGASIDSSASSSLR	3	5	8	7	8	16	34	9	10	1	0					
INEAFEALK	8	8	26	22	36											
MQELGVDPFSYRPK	3	2	4	1	58	10	2	12	4	3						
QENLEGADFLR	12	7	4	39	23	6	11									
SAISYIER	7	17	56	20												
TCSSQWPSVSDHSR	12	2	9	2	4	53	12	1	2	3						
GLVITAK	12	25	63													
LPCVEEVVEK	13	10	7	21	21	28										
LQDLLHR	11	20	69													
TVANPNQR	2	31	12	55												

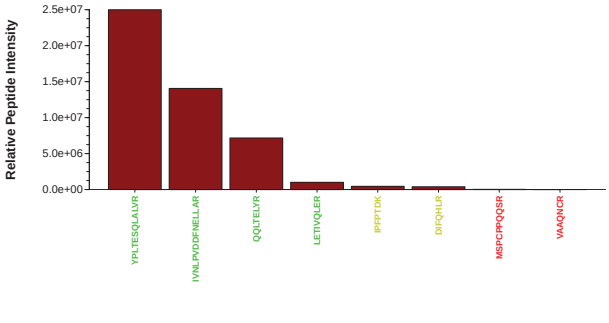
NFATC3



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
ESSLSPSPASSISSR	3	1	2	4	1	14	4	53	16	1	0					
NYEGTCEIPESK	2	62	5	2	4	2	12	11								
VLSLQIASIPVECSQR	1	3	1	1	51	6	11	18	7	1						
DHLYLPLEPSYR	0	14	2	1	45	15	14	10								
FTYTPVLMK	8	2	29	16	46											
DMSQISVSQGAGVSR	1	10	4	14	5		11	39	12	2	1					
TVATASQEIIIASTK	12	15	11	9	4	8	5	17	7	4	7					
VHIPQPSGK	1	25	3	48	22											
YLRPHAFYQVHR	9	1	87	3	0	0	0	0								

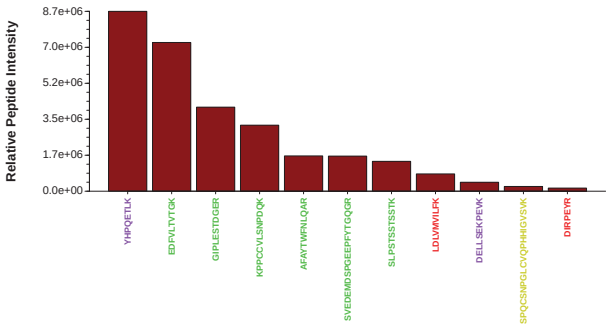
NFE2



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
IVNLPVDDFNELLAR	4	4	1	2	7	6	8	4	61	2						
LETIVQLER	5	15	33	11	36											
QQLTELYR	19	21	34	26												
YPLTESQLALVR	5	8	6	4	17	14	29	17								
DIFQHLR	20	23	57													
IPFPTDK	5	64	31													

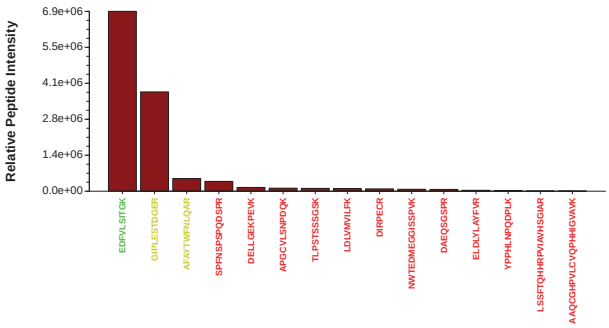
NFIA



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AFAYTWFNLQAR	6	5	12	23	21	22	7	5								
EDFVLTVTGK	12	8	25	39	10	6										
GIPLESTDGER	14	3	4	19	35	13	13									
KPPCCVLSNPDQK	4	38	6	12	8	4	4	3	21							
SLPSTSSTSSTK	2	3	3	6	11	21	21	33								
SVEDEMDSPGEEPFYTGQGR	2	14	17	7	1	26	8	1	3	19	4					
SPQCSNPGLCVQPHHIGVSVK	10	3	28	11	12	3	23	2	4	5	1					
DELLSEKPEVK	0	27	11	6	37	16	3									
YHPQETLK	3	4	1	92												

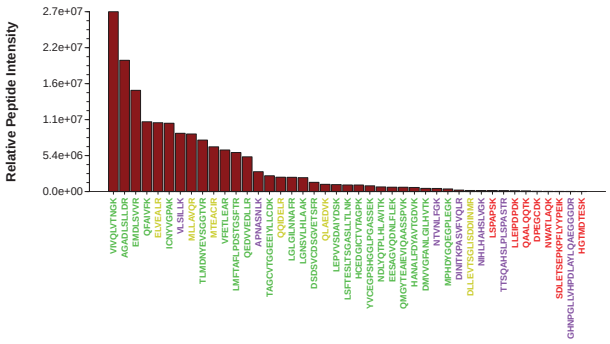
NFIC



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
EDFVLSITGK	8	5	29	40	11	7										
AFAYTWFNLQAR	5	5	12	23	22	22	7	5								
GIPLESTDGER	11	3	3	20	34	12	17									

NFKB1



(Page 1)

Relative Contribution of Transition to Signal (%)

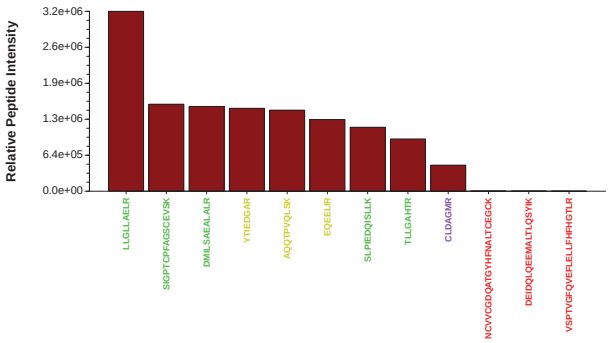
Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AGADLSLLDR	10	14	43	15	14	4										
DMVVGFANLGILHVTK	7	13	9	3	14	4	8	13	5	19	6	0				
DSDSVCDSGVETSFR	7	11	14	3	10	13	11	26	3	1	0					
EESAGVQDNLFLEK	13	14	3	6	12	26	5	14	4	3						
EMDLSVVR	13	55	11	21												
HANALFDYAVTGDVK	3	7	17	7	6	11	9	16	7	6	10					
HCEDGICTVTAGPK	14	9	13	3	5	13	2	16	9	15						
ICNYVGPAK	7	27	12	9	45											
LEPVVSDAIYDSK	4	7	3	6	5	21	31	4	17							
LGLGILNNAFR	3	5	19	40	7	24	2									
LGNSVLHLAAK	11	10	19	33	13	11	4									
LMFTAFLPDSTGSFTR	1	1	2	1	9	1	56	12	12	3	2					
LSFTESLTSGASLLTLNK	5	20	8	4	8	3	11	14	17	4	5	1				
MPHDYGQEGPLEGK	7	1	9	21	5	1	14	20	16	4						
NDLYQTPLHLAVITK	12	7	7	5	4	1	33	18	7	6	0					
QEDVVEDLLR	9	9	27	36	6	13										
QFAIVFK	59	14	27													
QMGYTEAIEVIQAASSPVK	9	7	9	6	8	9	11	7	17	8	7	2				
TAGCVTGGEEIYLLCDK	21	18	11	12	4	4	2	5	11	11	2					
TLMDNYEVSGGTVR	1	2	9	24	16	19	8	5	8	6						
VFETLEAR	7	8	26	60												
VIVQLVTNGK	5	13	14	21	14	34										
YVCEGPSHGGLPGASSEK	5	9	2	3	43	2	4	15	4	1	5	6	1			
DLLEVTSGLISDDIINMR	15	12	7	6	7	21	16	4	4	4	4					
ELVEALR	27	49	24													

(Page 2)

Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
MLLAVQR	10	26	64													
MTEACIR	8	29	63													
QLAEDVK	23	21	57													
QQIDELR	18	47	35													
APNASNLK	3	16	22	59												
DINITKPASVFVQLR	2	2	3	1	3	2	64	8	13	1	0					
GHNPGLLVHPDLAYLQAEGGGDR	0	1	17	6	12	7	2	2	3	22	8	21				
NIHLHAHSLVGK	12	7	18	9	8	30	11	5								
NTVNLFGK	25	11	59	5												
TTSQAHSLPLSPASTR	1	1	10	6	1	43	10	15	8	4	0	0				
VLSILLK	5	3	91													

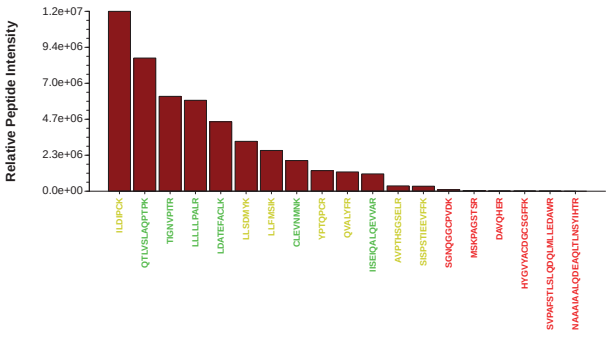
NR1I3



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
DMILSAEALALR	7	6	8	9	11	33	23	4								
LLGLLAELR	3	17	22	3	56											
SIGPTCPFAGSCEVSK	5	2	3	3	16	10	4	48	6	2	1	1				
SLPIEDQISLLK	5	10	4	5	11	36	9	19								
TLLGAHTR	5	5	40	50												
AQQTPVQLSK	6	6	2	37	23	26										
EQEELIR	31	27	43													
YTIEDGAR	17	13	23	47												
CLDAGMR	12	28	59													

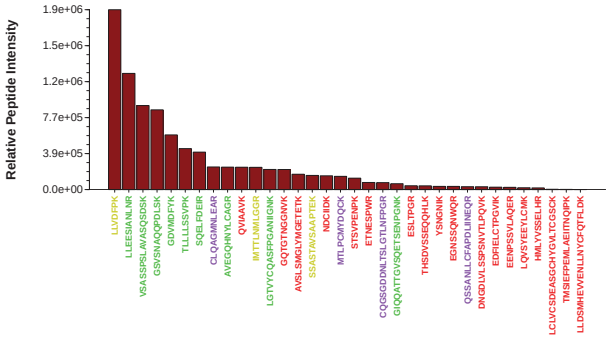
NR2E1



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
CLEVNMNK	5	16	22	56												
IISEIQALQEVVAR	10	6	7	12	13	16	20	7	2	6						
LDATEFACLK	7	11	22	20	16	23										
LLLLLPALR	0	29	21	20	29											
QTLVSLAQPTPK	1	13	4	18	8	36	11	9								
TIGNVPITR	0	27	4	4	64											
AVPTHSGGSELR	2	2	13	29	14	7	32									
ILDIPCK	32	10	58													
LLFMSIK	5	6	89													
LLSDMYK	6	3	91													
QVALYFR	42	14	44													
SISPSTIEEVFFK	15	4	5	17	10	7	10	19	14							
YPTQPCR	45	14	42													

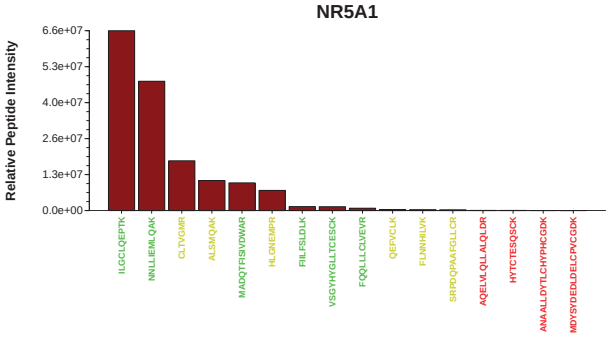
NR3C1



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
AVEGQHNYLCAGR	17	18	38	8	4	12	1	1	0							
GDVMDFYK	9	26	47	18												
GIQQATTGVSQETSENPGNK	3	48	10	1	8	5	2	2	13	1	6	2				
GSVSNAQQPDLSK	6	2	46	6	9	12	8	12	1							
LGTVYCQASFPGANIGNK	16	10	2	4	2	2	39	8	8	7	1	1				
LLEESIANLNR	4	16	26	9	14	11	20									
SQELFDEIR	6	12	28	21	34											
TLLLLSSVPK	2	3	14	24	24	33										
VSASSPSLAVASQSDSK	1	5	2	10	16	10	13	3	4	28	4	2	0			
IMTTLNMLGGR	9	10	5	18	13	9	37									
LLVDFPK	11	10	80													
SSASTAVSAAPTEK	2	23	9	7	24	15	11	4	3	2						
CLQAGMNLEAR	5	3	7	5	29	32	19									
CQGSGDDNLTSLGTLNFPGR	31	11	10	5	3	16	5	9	8	1	1					
MTLPCMYDQCK	4	8	8	11	7	60	3									
QSSANLLCFAPDLIINEQR	0	11	11	4	5	3	38	13	9	7						

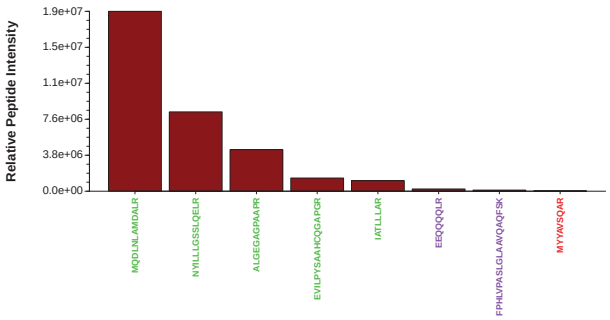
NR5A1



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
FIILFSLDLK	8	70	2	1	9	10										
FQQLLLCLVEVR	11	7	6	16	23	17	10	10								
ILGCLQEPTK	13	5	6	9	5	62										
MADQTFISIVDWAR	9	11	9	6	27	16	13	7	1	1						
NNLLIEMLQAK	5	6	8	18	32	24	8									
VSGYHYGLLTCESCK	12	4	8	14	11	4	27	17	3							
ALSMQAK	3	6	92													
CLTVGMR	18	15	67													
FLNNHILVK	14	11	11	14	50											
HLGNEMPR	9	2	5	84												
QEFVCLK	50	22	27													
SRPDQPAAFGLLCR	2	1	4	4	3	4	25	44	2	11						

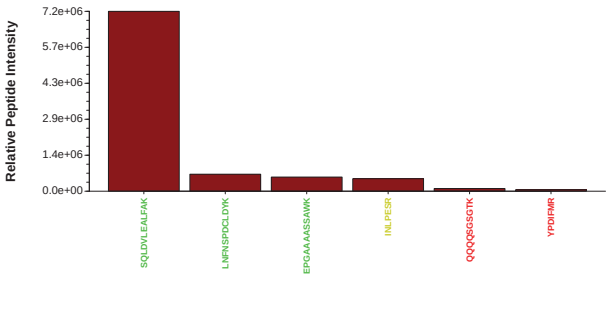
OLIG1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ALGEGAGPAAPR	5	1	13	24	7	25	4	21								
EVILPYSAAHCQGAPGR	15	2	6	3	8	9	7	6	7	3	34	1				
IATLLLAR	10	28	15	47												
MQDLNLAMDALR	9	6	10	24	10	15	6	19								
NYILLLGSSLQELR	3	4	4	5	9	40	16	10	8	2						
EEQQQQLR	31	33	25	11												
FPHLVPASLGLAAVQAQFSK	8	4	7	7	6	8	6	2	8	4	3	1	35			

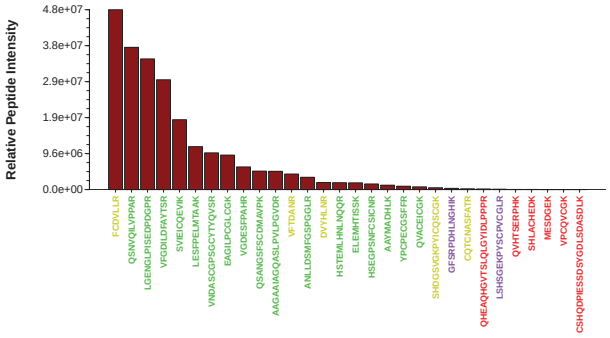
OTX1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
EPGAAAASSAWK	2	4	11	23	33	22	1	4								
LNFNSPDCLDYK	8	3	9	3	34	19	14	11								
SQLDVLEALFAK	8	5	7	10	19	8	18	24								
INLPESR	4	64	32													

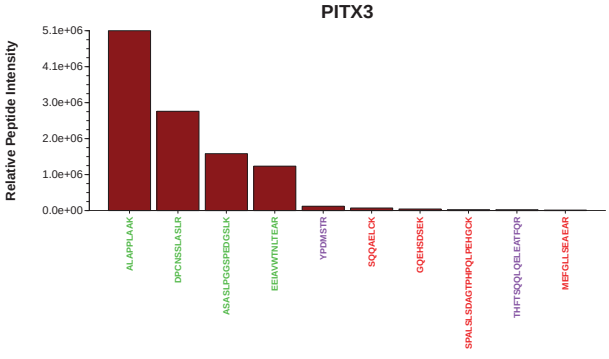
PATZ1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AAGAAIAGQASLPVLPGVDR	0	1	29	6	1	29	4	9	6	2	7	5	1			
AAYMADHLK	9	6	34	29	21											
ANLLDSMFGSPGGLR	1	1	18	6	19	19	6	10	14	4	0					
EAGILPCGLCGK	7	1	4	3	61	20	1	3								
ELEMHTISSK	20	9	20	21	16	16										
HSEGPSNFCSCNR	11	4	10	12	9	5	3	11	21	14						
HSTEMLHNLNQQR	3	8	10	21	27	11	8	4	8							
LESFPELMTAAK	2	6	4	5	3	51	5	25								
LGENGLPISEDPDGPR	17	0	20	3	1	6	1	48	1	1	0	0				
QSANGSFSCDMAVPK	16	7	9	4	7	19	15	6	9	5	3					
QSNVQILVPPAR	1	49	8	24	7	7	2	2								
QVACEICGK	25	15	19	11	30											
SVIEICQEVIK	4	5	5	26	26	23	11									
VFGDILDFAYTSR	5	8	8	14	20	23	6	2	13							
VGDESFPAHR	4	50	14	20	6	4										
VNDASCGPSGCYTYQVSR	12	18	12	10	8	5	9	2	12	13						
YPCPECGSFFR	2	2	8	10	5	56	16									
CQTCNASFATR	11	8	16	12	10	15	28									
DVYHLNR	27	37	36													
FCDVLLR	10	20	70													
SHDGSVGKPYICQSCGK	6	14	4	14	5	2	40	3	11	2	1					
VFTDANR	22	11	66													
GFSRPDHLNGHIK	5	16	12	7	49	1	10	0	0							
LSHSGEKPYSCPVCGLR	2	7	0	7	5	4	3	58	11	2						

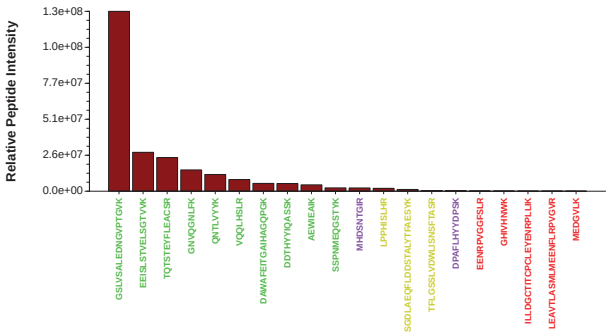
PITX3



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ALAPPLAAK	2	1	14	42	42											
ASASLPGGSPEDGSLK	0	5	3	0	7	2	3	5	72	1	1	0				
DPCNSSLASLR	12	16	9	14	19	19	10									
EEIAVWTNLTEAR	3	6	3	9	17	39	12	9	1							
THFTSQQLQELEATFQR	13	11	23	15	5	5	10	10	5	2						
YPDMSTR	11	32	57													

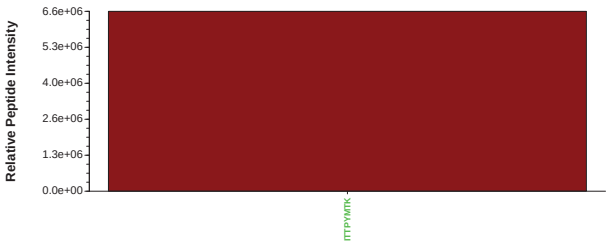
PLEK2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AEWIEAIK	5	12	19	64												
DAWAFEITGAIHAGQPGK	27	1	5	7	10	4	3	13	14	7	5	3	1			
DDTHYYIQASSK	13	13	8	10	24	28	3	0								
EEISLSTVELSGTVVK	3	3	9	10	7	16	8	10	23	4	7	1				
GNVQGNLFK	4	3	52	28	14											
GSLVSALEDNGVPTGVK	4	2	54	3	3	3	6	8	6	3	7	0	0			
QNTLVYYK	44	34	11	12												
SSPNMEQGSTYK	5	4	17	12	17	19	14	11								
TQTSTEYFLEACSR	6	16	13	12	14	14	6	5	5	7						
VQQLHSLR	15	24	19	42												
LPPHISLHR	1	13	12	28	46											
SGDLAEQFLDDSTALYTFAESYK	13	9	15	10	18	15	8	6	3	3	2					
TFLGSSLVDWLISNSFTASR	5	8	5	5	5	28	15	11	7	8	3					
DPAFLHYYDPSK	62	5	4	7	12	6	3	0								
MHDSNTGIR	1	3	3	24	69											

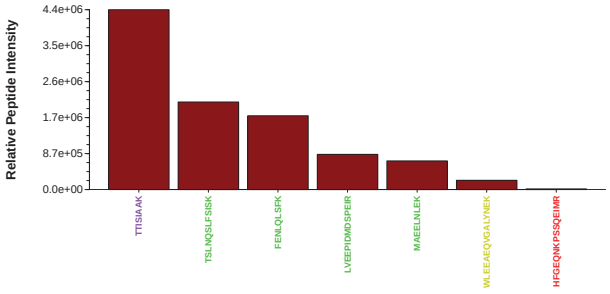
POLR2F



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ITTPYMTK	8	7	40	45												

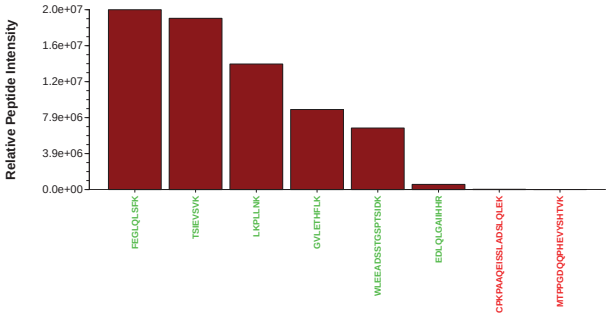
POU1F1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
FENLQLSFK	14	12	13	10	50											
LVEEPIDMDSPEIR	0	9	15	9	8	13	3	39	3	1						
MAEELNLEK	5	11	14	20	49											
TSLNQSLFSISK	4	17	14	8	22	10	20	6								
WLEEAEQVGALYNEK	9	14	9	4	31	11	5	8	6	2	0					
TTISIAAK	4	11	24	61												

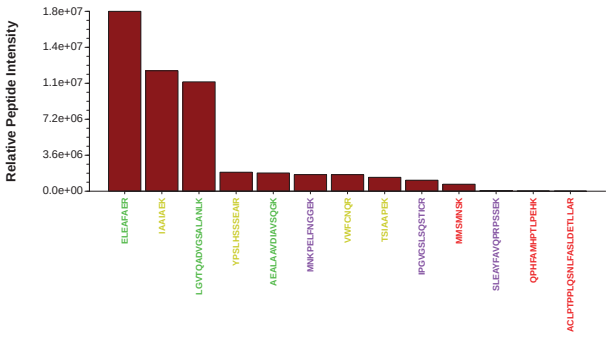
POU3F4



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
EDLQLGAIIHHR	12	11	8	6	39	18	4	0								
FEGLQLSFK	8	7	9	3	72											
GVLETHFLK	8	7	23	28	34											
LKPLLNK	4	1	95													
TSIEVSVK	6	10	29	55												
WLEEADSSTGSPTSIDK	4	7	1	21	6	13	9	9	11	11	7	2				

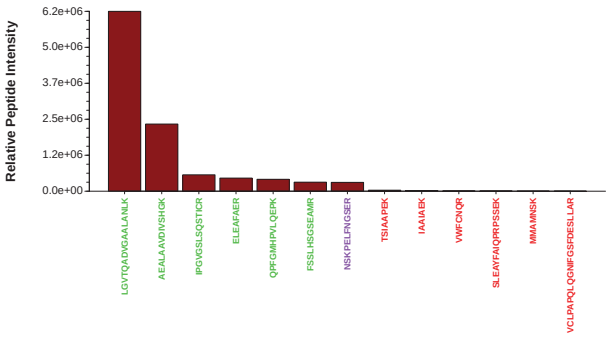
POU4F1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AEALAAVDIAVSQGK	3	16	7	8	5	14	14	17	12	2	1					
ELEAFAER	29	30	23	18												
LGVTQADVGSALANLK	6	9	4	4	5	25	8	15	17	5	3	0				
IAAIAEK	10	8	82													
TSIAAPEK	18	13	33	35												
VWFCNQR	4	17	78													
YPSLHSSSEAIR	5	6	3	9	29	29	6	13								
IPGVGSLSQSTICR	2	4	7	6	20	7	8	31	3	12						
MNKPELFNGGEK	1	4	4	6	6	5	72	3								
SLEAYFAVQPRPSSEK	0	0	4	1	30	16	13	19	14	1	1					

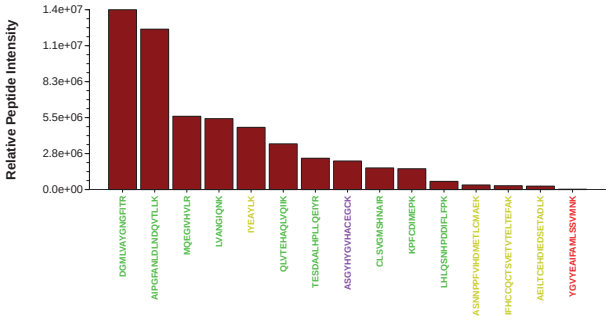
POU4F3



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AEALAAVDIVSHGK	4	13	10	5	13	13	19	19	3	2						
ELEAFAER	24	32	26	18												
FSSLHSGSEAMR	6	1	2	12	38	34	3	4								
IPGVGSLSQSTICR	2	4	7	5	21	8	8	28	3	13						
LGVTQADVGAALANLK	5	9	5	6	4	25	8	14	16	5	3	0				
QPFGMHPVLQEPK	6	6	8	2	50	13	2	6	7							
NSKPELFNGSER	1	3	3	5	7	2	80	1								

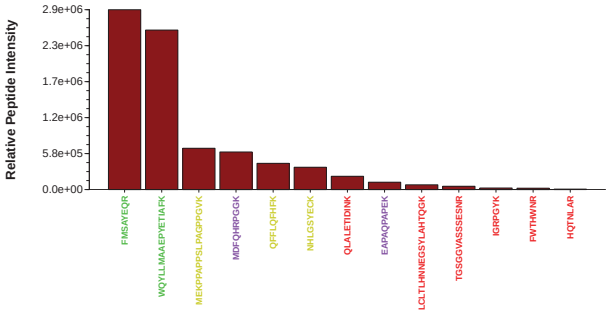
PPARA



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AIPGFANLDLNDQVTLLK	21	15	3	6	4	9	10	18	5	6	3					
CLSVGMSHNAIR	3	5	5	11	6	37	10	24								
DGMLVAYGNGFITR	4	2	3	3	19	18	35	13	2	0						
KPFCDIMEPK	10	8	19	7	31	26										
LHLQSNHPDDIFLFPK	35	9	7	12	6	1	22	3	2	4						
LVANGIQNK	4	2	8	12	74											
MQEGIVHVLR	5	16	22	4	20	33										
QLVTEHAQLVQIIK	9	17	8	6	8	16	17	5	11	4						
TESDAALHPLLQEIYR	3	2	3	4	2	59	19	5	2	0						
AEILTCEHDIEDSETADLK	15	11	12	4	22	7	6	8	8	7						
ASNNPPFVIHDMETLCMAEK	16	13	17	5	9	3	7	9	14	7						
IFHCCQCTSVETVTELTEFAK	18	10	19	11	4	15	3	7	8	2	2					
IYEAYLK	6	18	75													
ASGYHYGVHACEGCK	16	5	10	14	16	3	19	14	4	0						

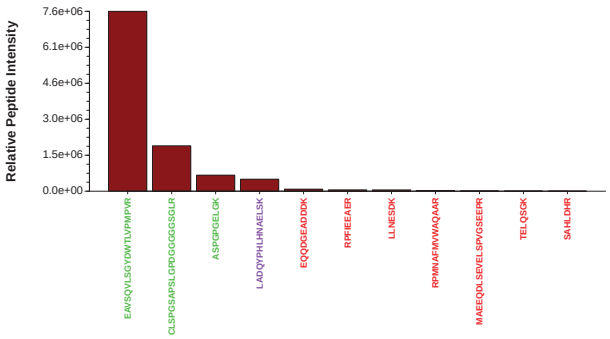
SF3A2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
FMSAYEQR	5	13	9	73												
WQYLLMAAEPYETIAFK	7	20	4	1	1	19	9	13	12	10	3					
MEKPPAPPSLPAGPPGVK	1	4	8	5	1	17	2	2	6	33	3	8	11			
NHLGSYECK	4	3	6	51	37											
QFFLQFHFK	14	24	19	19	24											
EAPAQPAPEK	55	4	24	4	4	9										
MDFQHRPGGK	5	19	6	17	3	50										

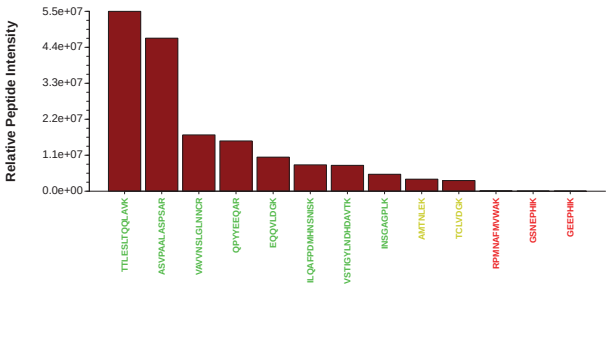
SOX10



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ASPGPGELGK	7	1	4	27	29	32										
CLSPGSAPSLGPDGGGGGSGLR	2	2	1	2	2	2	16	1	7	21	4	2	33	4	1	0
EAVSQVLSGYDWTLVPMPVR	7	1	62	12	3	3	4	3	2	3						
LADQYPHLHNAELSK	13	5	6	8	15	7	5	39	3	0						

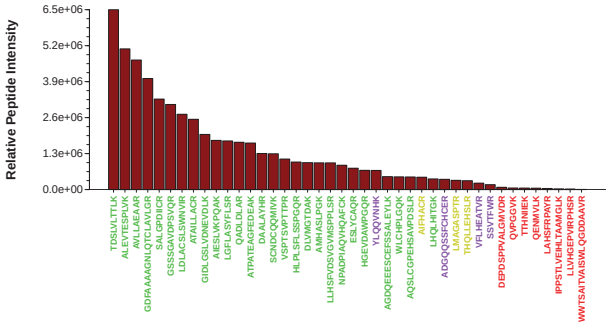
SOX5



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ASVPAALASPSAR	0	8	15	22	13	14	7	21	0							
EQQVLDGK	28	41	19	11												
ILQAFPDMHNSNISK	9	2	3	5	5	8	3	50	9	6	0					
INSGAGPLK	5	13	7	13	62											
QPYYEEQAR	9	24	32	15	19											
TTLESLTQQLAVK	12	7	6	7	22	8	22	11	5							
VAVVNSLGLNNCR	3	10	5	32	10	11	19	8	1							
VSTIGYLNDHDAVTK	4	10	5	10	5	15	15	8	25	2	1					
AMTNLEK	5	7	88													
TCLVDGK	19	19	62													

SREBF2

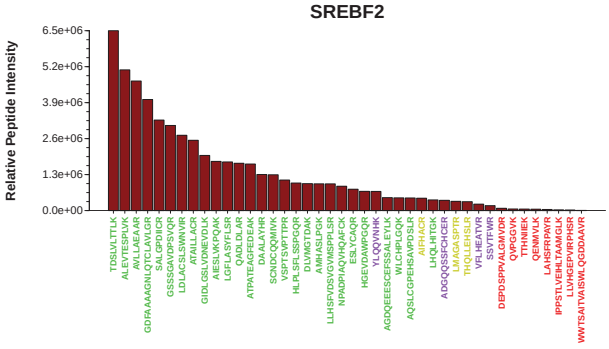


(Page 1)

Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ACDQEEESCEFSSALEYLK	17	16	10	7	9	16	9	6	6	7						
AIESLVKPQAK	1	36	15	19	6	11	13									
ALEVTESPLVK	1	11	13	11	32	18	14									
AMHASLPGK	43	4	12	28	14											
AQSLCGPEHSAVPDSLR	18	1	39	4	5	10	6	1	6	7	4					
ATAILLACR	15	27	36	10	12											
ATPATEAGFEDEAK	4	5	4	5	22	19	9	19	5	7						
AVLLAEAAR	5	9	39	23	24											
DAALAYHR	18	46	22	13												
DLVMGTDAK	1	1	21	43	32											
ESLYCAQR	21	45	28	6												
GDFAAAAGNLQTCLAVLGR	18	13	9	3	5	9	9	5	4	15	7	3				
GIDLGSLVDNEVDLK	9	5	2	7	18	23	9	8	17	1	1					
GSSSGAVDPSVQR	2	2	53	17	12	5	7	2	1							
HGEVDAWPGQR	1	26	9	29	15	11	10									
HLPLSFLSSPGQR	0	5	4	5	4	2	6	1	73							
LDLACSLSWNVIR	3	9	10	23	8	17	19	9	1							
LGFLASYFLSR	3	8	9	26	39	13	2									
LHQLHITGK	21	13	13	17	36											
LLHSFVDSVGVMSPPLSR	0	2	22	11	9	3	19	4	11	9	7	2				
NPADPIAQVHQAFCK	6	8	6	15	8	5	12	3	31	5	1					
QADLDLAR	27	41	12	19												
SALGPDIICR	11	11	7	26	40	6										
SCNDCQQMIVK	15	15	9	7	13	12	28									
TDSLVLTTLK	5	11	23	29	7	26										
VSPTSVPTTPR	4	3		16	42	16	19									

SREBF2

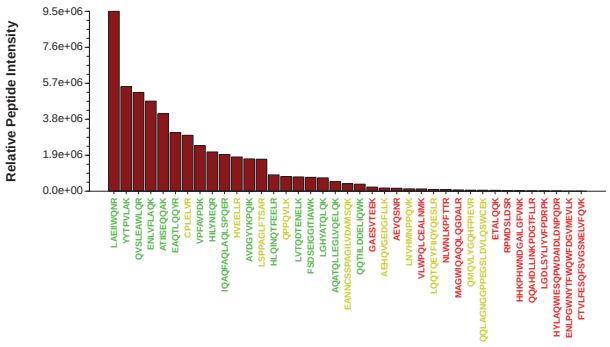


(Page 2)

Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
WLCHPLGQK	10	6	39	11	34											
AIFHACR	30	28	42													
LMAGASPTR	7	8	5	19	61											
THQLLEHSLR	5	14	10	15	20	35										
ADGQQSSFCHCER	14	15	13	12	13	25	7	0	0							
SSVTFWR	17	56	27													
VFLHEATVR	2	20	33	28	16											
YLQQVNHK	13	38	16	32												

STAT5A

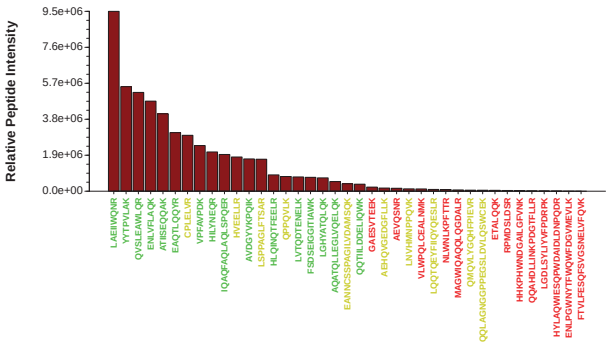


(Page 1)

Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AQATQLLEGLVQELQK	7	11	10	13	3	19	14	14	6	2	1					
ATIISEQQAK	2	4	7	48	30	8										
AVDGYVKPQIK	1	39	15	16	4	7	19									
EAQTLQQYR	17	39	17	23	4											
ENLVFLAQK	11	14	48	20	7											
FSDSEIGGITIAWK	14	5	8	3	8	36	17	3	3	3						
HILYNEQR	1	5	16	79												
HLQINQTFEELR	4	3	4	6	4	19	20	38								
IQAQFAQLAQLSPQER	1	29	23	8	8	13	6	5	5	3						
LAEIIWQNR	10	37	31	12	11											
LGHYATQLQK	8	4	12	33	41	1										
LVTQDTENELK	2	6	4	13	27	7	40									
QQTIILDDELIQWK	12	11	5	4	5	15	24	18	2	3						
QVSLEAWLQR	10	14	24	18	5	29										
VPFAVPDK	39	8	19	34												
YYTPVLAK	9	3	28	59												
AEHQVGEDGFLLK	8	1	19	8	1	26	27	7	2							
CPLELVR	24	35	42													
EAANCSSPAGILVDAMSQK	11	6	8	16	8	16	2	6	1	19	4	3				
HVEELLR	10	13	78													
LNVHMNPPQVK	1	8	49	12	16	11	3									
LQQTQEYFIIQYQESLR	6	6	7	14	17	20	13	12	5							
LSPPAGLFTSAR	2	5	11	3	16	17	35	11								
QMQVLYGQHFPIEVR	3	1	35	12	9	2	19	15	3	2						
QPPQVLK	27	8	65													

STAT5A

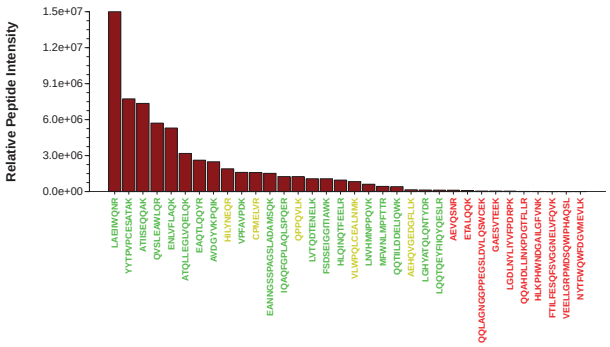


(Page 2)

Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
QQLAGNGGPPEGSLDVLQSWCEK	15	10	33	12	11	6	1	1	4	4						

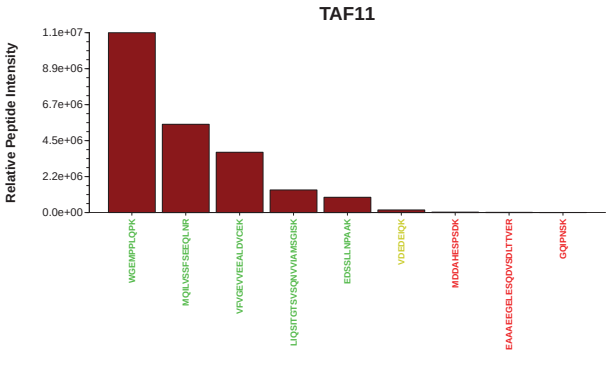
STAT5B



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
ATIISEQQAK	2	4	7	49	30	8										
ATQLLEGLVQELQK	6	9	10	14	4	24	20	10	3	0						
AVDGYVKPQIK	1	34	15	18	4	8	19									
EAANGSSPAGSLADAMSQK	12	7	8	7	8	4	2	10	2	27	7	2	3			
EAQTLQQYR	16	39	16	23	6											
ENLVFLAQK	10	14	49	20	8											
FSDSEIGGITIAWK	13	4	8	2	9	37	17	4	3	3						
HLQINQTFEELR	5	3	4	6	5	20	20	36								
IQAQFGPLAQLSPQER	1	22	17	7	8	7	1	12	18	7	1					
LAEIIWQNR	9	37	31	12	11											
LGHYATQLQNTYDR	3	5	8	7	12	7	15	30	13							
LNVHMNPPQVK	1	7	41	17	17	13	3									
LQQTQEYFIIQYQESLR	5	7	5	15	17	24	13	8	5							
LVTQDTENELK	2	6	4	12	29	8	40									
MFWNLMPFTTR	2	1	41	29	11	12	6									
QQTIILDDELIQWK	11	8	4	5	6	18	25	17	3	3						
QVSLEAWLQR	10	14	23	18	5	30										
VPFAVPDK	38	8	19	36												
YYTPVPCESATAK	1	1	3	6	2	45	3	33	7							
AEHQVGEDGFLLK	6	2	18	5	2	31	28	8	1							
CPMELVR	22	30	48													
HILYNEQR	1	4	15	80												
QPPQVLK	24	11	65													
VLWPQLCEALNMK	6	4	6	3	8	14	3	52	2							

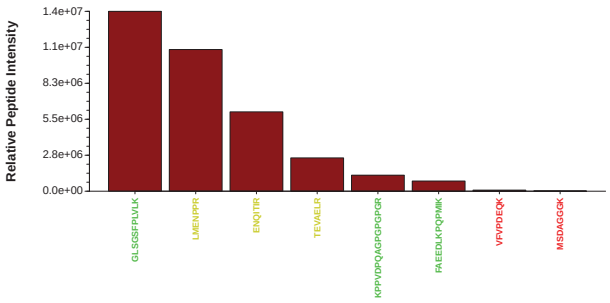
TAF11



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
EDSSLLNPAAK	1	26	18	34	10	5	6									
LIQSITGTSVSQNVVIAMSGISK	2	7	15	11	24	12	7	2	4	2	11	3				
MQILVSSFSEEQLNR	4	6	5	4	11	8	10	32	13	7						
VFVGEVVEEALDVCEK	16	11	13	8	9	7	13	14	5	1	2					
WGEMPPLQPK	2	1	7	73	10	7										
VDEDEIQK	10	10	23	58												

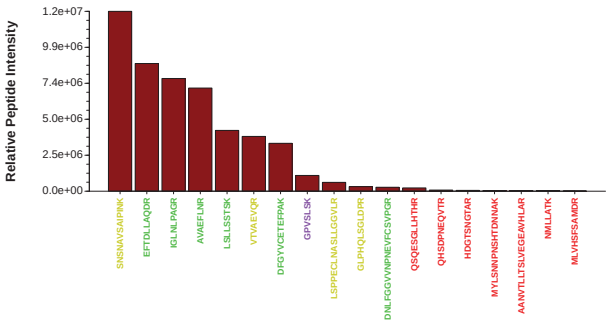
TEF



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
FAEEDLKPQPMIK	2	30	1	38	11	7	6	3	2							
GLSGSFPLVLK	4	1	24	12	10	14	35									
KPPVDPQAGPGPGPGR	1	0	2	1	3	5	3	1	68	4	2	10				
ENQITIR	63	25	12													
LMENPPR	22	23	55													
TEVAELR	5	29	66													

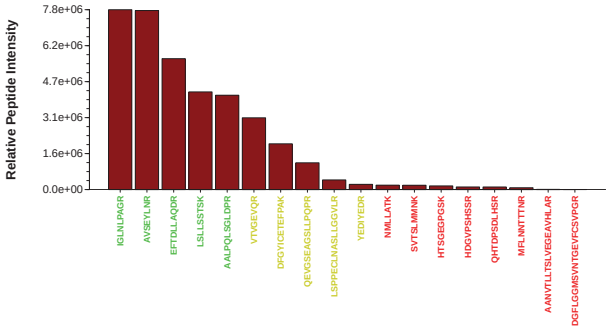
TFAP2A



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AVAEFLNR	6	26	12	56												
DFGYVCETEPPAK	24	11	3	6	5	26	20	1	4							
DNLFGGVVNPNEVFCSVPGR	51	5	7	7	6	3	1	1	16	4	0					
EFTDLLAQDR	9	38	26	9	8	11										
IGLNLPAGR	2	57	13	24	5											
LSLLSSTSK	2	6	30	28	34											
GLPHQLSGLDPR	10	2	10	21	23	23	7	4								
LSPPECLNASLLGGVLR	5	4	15	9	6	16	12	15	7	8	1	0				
SNSNAVSAIPINK	1	54	5	4	19	10	3	2	2							
VTVAEVQR	8	15	34	43												
GPVSLSK	6	44	50													

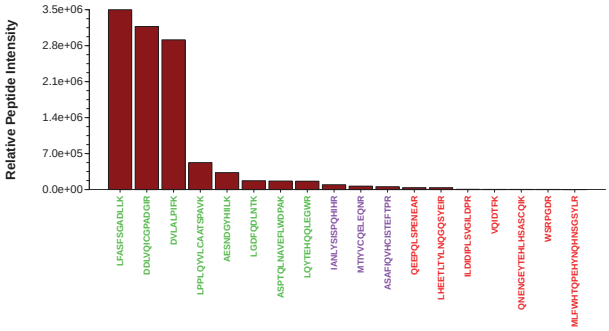
TFAP2B



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AALPQLSGLDPR	3	1	9	21	15	4	45	1								
AVSEYLNR	7	20	7	66												
EFTDLLAQDR	9	37	27	8	7	12										
IGLNLPAGR	1	57	13	24	5											
LSLLSSTSK	2	6	30	29	34											
DFGYICETEPPAK	24	12	3	6	6	26	19	2	3							
LSPPECLNASLLGGVLR	4	4	17	11	6	13	11	17	7	7	2	1				
QEVGSEAGSLLPQPR	1	41	13	5	5	12	8	3	2	7	4					
VTVGEVQR	5	5	40	50												
YEDIYEDR	5	29	12	53												

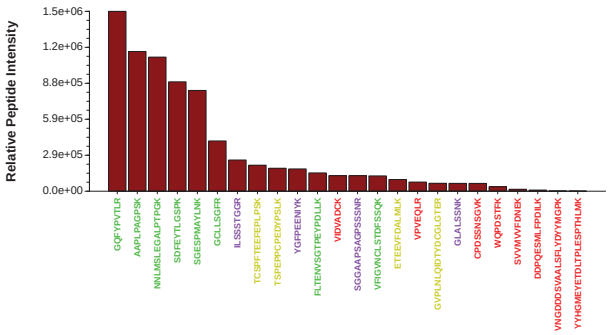
TFCP2L1



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AESNDGYHIILK	18	7	20	4	20	10	5	16								
ASPTQLNAVEFLWDPAK	24	12	8	4	9	12	7	6	11	7						
DDLVQICGPADGIR	11	3	0	6	19	26	16	16	2	0						
DVLALPIPK	0	19	14	30	37											
LFASFSGADLLK	3	2	1	9	17	11	19	38								
LGDFQDLNTK	12	17	19	21	20	11										
LPPLQYVLCAATSPAVK	0	24	7	8	8	6	13	13	9	9	3					
LQYTEHQQLEGWR	14	9	10	10	14	20	8	10	5							
ASAFIQVHCISTEFTPR	5	8	2	5	14	7	21	27	8	4						
IANLYSISPQHIHR	4	3	0	16	30	7	23	17	1	0						
MTIYVCQELEQNR	4	19	8	9	5	32	16	7	0							

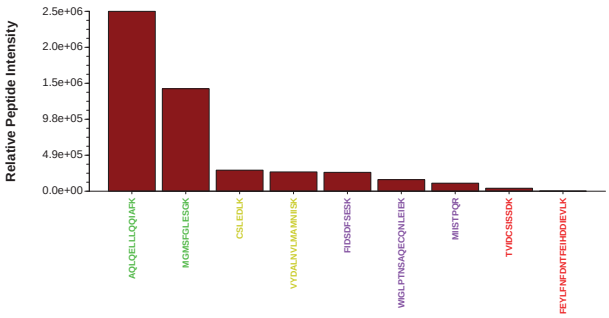
TFCP2L4



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AAPLPAGPSK	2	6	3	68	4	16										
FLTENVSGTPEYPDLLK	3	1	22	7	0	31	3	8	19	3	2					
GCLLSGFR	7	27	33	33												
GQFYPVTLR	2	3	47	18	29											
NNLMSLEGALPTPGK	27	3	20	7	2	9	8	4	11	8	1					
SDFEYTLGSPK	12	8	6	17	31	16	10									
SGESPMAYLNK	11	11	8	5	43	21	1									
VFIGVNCLSTDFSSQK	8	10	7	7	8	24	8	6	14	3	6					
ETEEVFDALMLK	12	5	8	11	39	14	6	6								
GVPLNLQIDTYDCGLGTER	1	14	3	4	16	7	13	13	17	13						
TCSPFTEEFEPLPSK	38	2	21	5	5	5	5	6	2	10						
TSPEPPCPEDYPSLK	1	14	21	2	2	17	3	8	31	0						
GLALSSNK	6	92	2	0												
ILSSSTGGR	2	7	15	7	69											
SGGAAPSAGPSSSNR	8	19	39	7	7	3	2	1	11	3	1					
YGFPEENIYK	2	4	6	6	78	3										

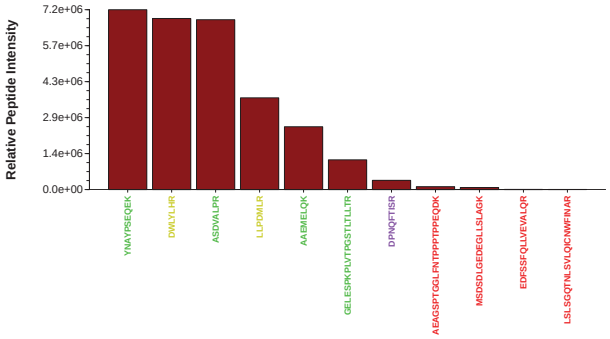
TFDP2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AQLQELLLQQIAFK	16	8	8	13	18	14	9	8	3	2						
MGMSFGLESGK	12	6	3	23	18	29	8									
CSLEDLK	19	30	51													
VYDALNVLMAMNIISK	11	5	9	9	14	16	21	5	7	2	1					
FIDSDFSESK	1	6	9	4	11	68										
MIISTPQR	6	5	32	56												
WIGLPTNSAQECQNLEIEK	22	19	8	9	2	13	11	6	6	4						

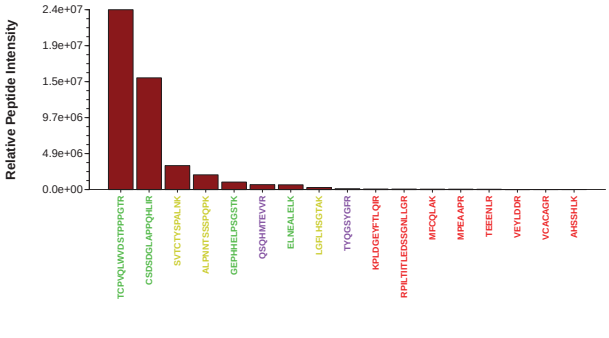
TGIF2



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AAEMELQK	10	13	31	46												
ASDVALPR	12	52	15	22												
GELESPKPLVTPGSTLTLLTR	2	2	2	1	1	1	2	32	22	8	2	25				
YNAYPSEQEK	2	1	3	65	12	17										
DWLYLHR	14	44	42													
LLPDMLR	14	14	72													
DPNQFTISR	9	25	36	19	12											

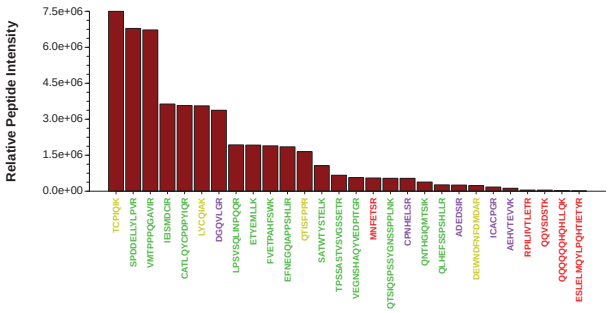
TP53



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
CSDSDGLAPPQHLIR	2	3	1	4	61	17	2	7	2	1	0					
ELNEALELK	12	15	31	11	31											
GEPHHELPSGSTK	27	7	23	20	18	3	0	1	0							
TCPVQLWVDSTPPPGTR	1	4	6	44	4	7	9	10	11	4	1					
ALPNNTSSSPQPK	1	10	7	13	22	12	15	8	13							
LGFLHSGTAK	0	3	31	36	23	5										
SVTCTYSPALNK	4	1	19	18	17	21	9	10								
QSQHMTEVVR	8	5	26	38	18	6										
TYQGSYGFR	8	5	5	33	47											

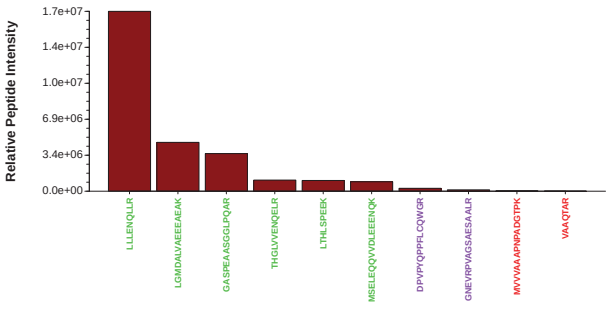
TP73L



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
CATLQYCPDPYIQR	3	1	44	1	18	15	12	5	1							
EFNEGQIAPPSHLIR	1	2	1	3	51	24	10	2	5	1	0					
ETYEMLLK	19	36	25	19												
FVETPAHFSWK	5	8	7	3	39	15	23									
IEISMDCR	7	9	10	32	42											
LPSVSQLINPQQR	1	25	20	16	9	6	15	3	5							
QLHEFSSPSHLLR	2	2	2	14	10	17	29	17	6							
QNTHGIQMTSIK	12	10	7	11	6	34	12	6								
QTSIQSPSSYGNSSPPLNK	1	7	50	5	3	2	9	3	2	2	12	4				
SATWTYSTELK	3	4	11	19	41	17	6									
SPDDELLYLPVR	19	17	24	19	11	4	3	3								
TPSSASTVSVGSSETR	0	2	4	18	8	25	9	9	14	4	4	3				
VEGNSHAQYVEDPITGR	2	1	67	4	4	6	6	5	5	1	0					
VMTPPPQGAVIR	1	1	5	3	21	31	31	9								
DEWNDFNFDMDAR	8	8	17	12	19	13	11	11	1							
LYCQAIK	5	6	90													
QTISFPPR	37	12	34	18												
TCPIQIK	3	2	95													
ADEDSIR	23	28	48													
AEHVTEVVK	7	17	26	36	13											
CPNHELSR	19	28	34	20												
DGQVLGR	40	55	5													
ICACPGR	22	15	63													

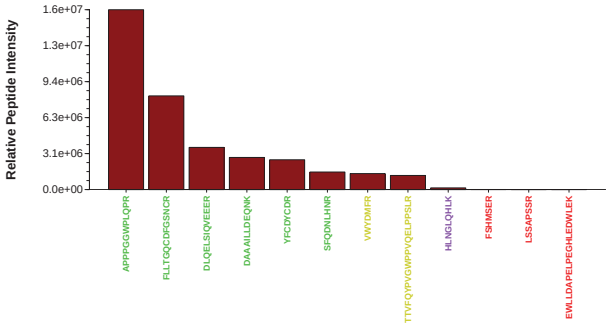
XBP1



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
GASPEAASGGLPQAR	1	23	5	6	14	23	13	10	3	3	0					
LGMDALVAEEEAEAK	3	5	3	5	9	33	25	10	4	3	0					
LLLENQLLR	4	3	19	25	49											
LTHLSPEEK	2	19	38	29	12											
MSELEQQVVDLEEENQK	9	7	12	10	8	19	21	10	3	1						
THGLVVENQELR	1	1	6	8	19	18	6	41								
DPVPYQPPFLCQWGR	2	3	5	4	2	7	72	3	2							
GNEVRPVAGSAESAALR	1	3	23	2	2	1	7	9	2	49	0	0				

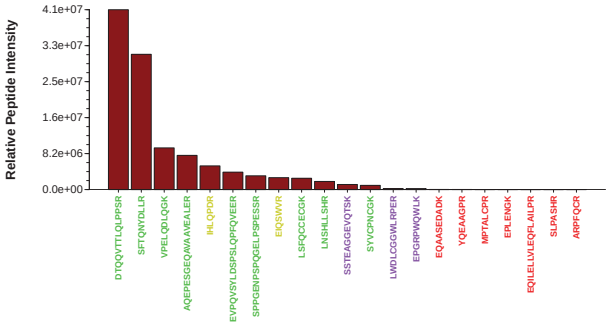
ZMAT5



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
APPPGGWPLQPR	1	1	14	2	2	7	26	48								
DAAAILLDEQNK	2	7	17	22	31	14	6	2								
DLQELSIQVEEER	6	10	9	14	11	30	12	8	1							
FLLTGQCDFGSNCR	4	5	17	15	8	14	4	18	12	3						
SFQDNLHNR	23	10	19	26	22											
YFCDYCDR	6	21	20	52												
TTVFQYPVGWPPVQELPPSLR	4	3	40	5	1	2	0	2	37	1	2					
VWYDMFR	12	12	76													
HLNGLQHLK	20	3	2	16	59											

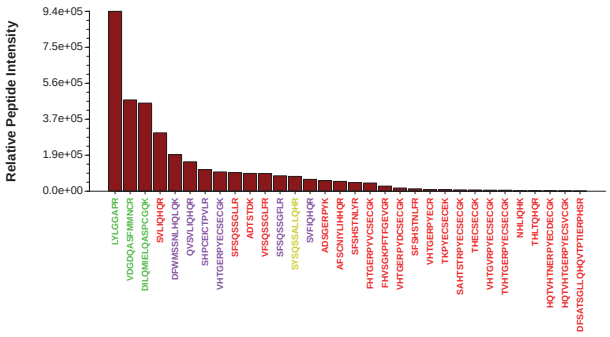
ZNF496



Relative Contribution of Transition to Signal (%)

Peptides	y3	y4	y5	y6	y7	y8	y9	y10	y11	y12	y13	y14	y15	y16	y17	y18
AQEPESGEQAVAAVEALER	3	9	12	8	11	22	10	9	6	2	4	3				
DTQQVTTLQLPPSR	2	57	7	6	4	4	14	5	2	0						
EVPQVSYLDSPSLQPFQVEER	9	8	5	1	40	8	2	2	17	8						
LNSHLLSHR	18	35	27	8	12											
LSFQCCECGK	14	6	15	40	12	12										
SFTQNYDLLR	12	14	14	30	10	19										
SPPGENPSPQGELPSPESSR	6	2	12	3	33	9	1	6	1	14	2	11				
SYVCPNCGK	7	4	45	23	20											
VPELQDLQGK	7	16	13	13	28	23										
EIQSWVR	14	52	34													
IHLQPDR	13	16	71													
EPGRPWQWLK	40	9	3	39	2	8										
LWDLCGGWLRPER	69	7	5	2	2	7	3	3	1							
SSTEAGGEVQTSK	10	6	5	2	9	37	23	6	2							

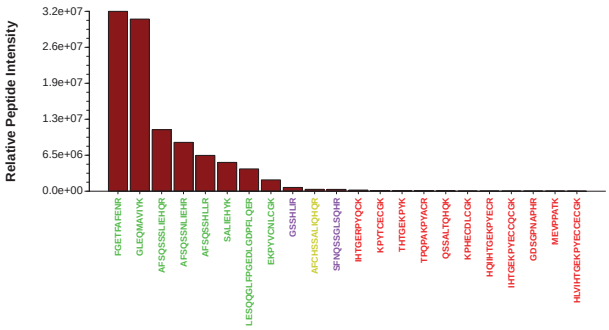
ZNF530



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
DILQMIELQASPCGQK	5	2	28	20	14	7	8	9	5	2	0					
LYLGGAPR	0	5	37	57												
VDGDQASFMMNCR	10	15	17	12	27	14	3	1	2							
SYSQSSALLQHR	9	17	11	13	14	22	5	8								
DFWMSSNLHQLQK	8	13	17	4	6	8	26	15	2							
QVSVLIQHQR	10	20	19	19	6	27										
SFSQSSGFLR	2	10	10	35	6	37										
SHPCEICTPVLR	7	3	5	10	14	7	6	47								
SVFIQHQR	10	35	25	29												
VHTGERPYECSECGK	1	8	4	5	3	0	14	65	0	0						

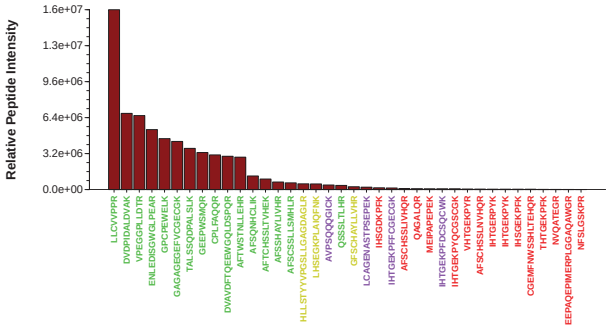
ZNF70



Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AFSQSSHLLR	**4**	**5**	**6**	**15**	**5**	**15**										
AFSQSSNLIEHR	19	14	6	7	14	25	4	10								
AFSQSSSLIEHQR	15	18	12	7	11	13	18	2	5							
EKPYVCNLCGK	8	3	5	16	13	7	49									
FGETFAFENR	8	20	30	21	17	4										
GLEQMAVIYK	22	12	19	20	14	13										
LESQQGLFPGEDLGDPFLQER	8	5	1	49	2	5	5	3	1	2	21					
SALIEHYK	10	24	36	29												
AFCHSSALIQHQR	10	16	9	9	7	14	26	8	1							
GSSHLIR	56	25	19													
SFNQSSGLSQHR	7	17	5	16	19	26	7	4								

ZNF74



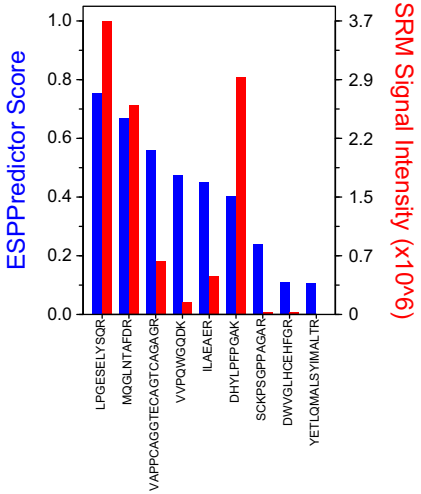
Relative Contribution of Transition to Signal (%)

Peptides	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
AFSCSSLLSMHLR	8	7	23	19	8	11	13	5	7							
AFSQNHCLIK	7	21	14	20	6	32										
AFSSHAYLIVHR	10	10	10	27	28	8	2	4								
AFTCHSSLTVHEK	25	8	10	4	11	21	14	3	3							
AFTWSTNLLEHR	10	11	6	9	14	33	11	6								
CPLFAQQR	6	36	38	20												
DVAVDFTQEEWGQLDSPQR	18	27	10	9	3	15	9	4	3	1						
DVDPIDALDVAK	4	6	4	8	23	7	29	18								
ENLEDISGWGLPEAR	1	33	5	17	5	10	19	4	5	2	0					
GAGAGEGEFVCGECGK	13	2	12	22	11	13	3	15	2	6	0	0				
GEEPWSMQR	7	26	13	49	5											
GPCPEWELK	3	10	3	70	15											
LLCVVPPR	28	27	18	27												
QSSSLTLHR	17	37	11	11	25											
TALSSQDPALSLK	12	4	2	26	9	5	14	24	5							
VPEGGPLLDTR	7	5	2	13	8	39	26									
GFSCHAYLLVHR	8	13	11	25	29	10	2	1								
HLLSTYYVPGSLLGAGDAGLR	1	22	1	2	2	4	3	2	1	1	49	8	4			
LHSEGKPLAIQFNK	11	9	5	6	2	36	3	7	5	16						
AVPSQQQGICK	3	16	6	10	7	21	37									
IHTGEKPFDCSQCWK	8	0	7	26	8	1	42	8	1							
IHTGEKPFFCGECGK	16	4	7	10	8	4	39	7	1	4						
LCAGENASTPSEPEK	22	3	3	23	5	12	6	9	2	6	9					

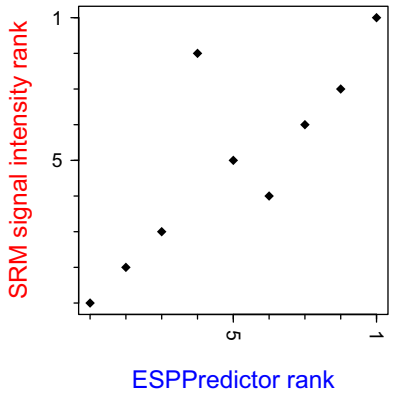
SUPPLEMENTARY DATA LEGENDS

Supplementary Data 2. Comparison of our empirical rankings with peptide rank predictions from the ESPPredictor algorithm

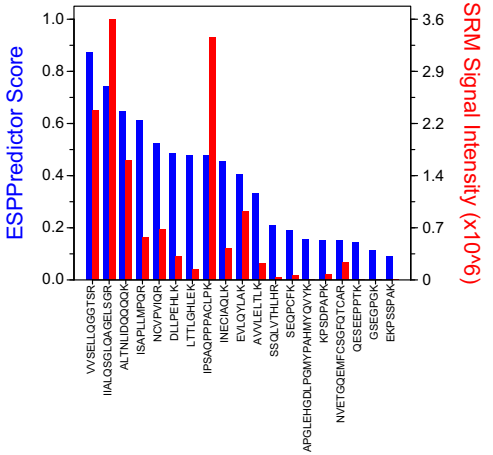
ATOH7
ESPPredictor vs. Empirical SRM intensities



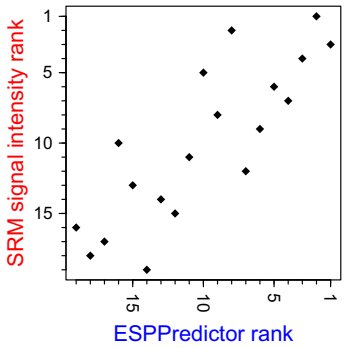
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.82)



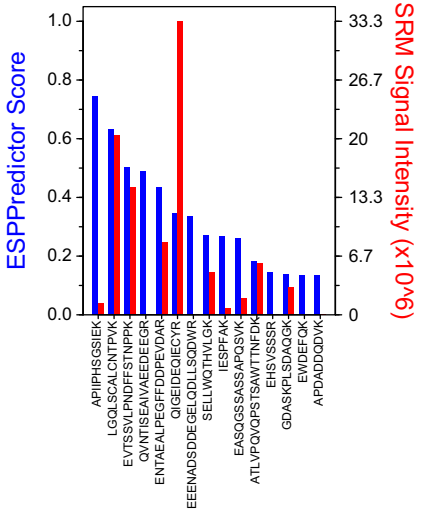
BHLHB2
ESPPredictor vs. Empirical SRM intensities



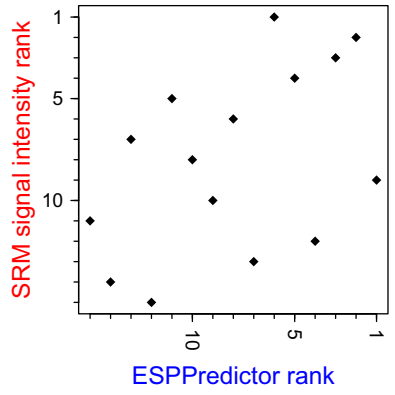
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.83)



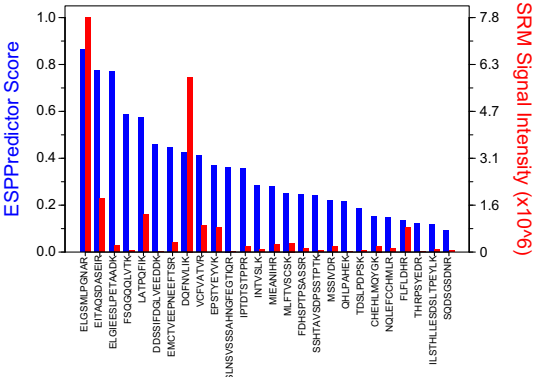
CCDC16
ESPPredictor vs. Empirical SRM intensities



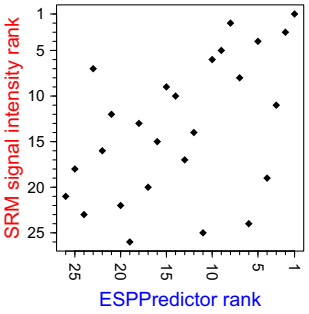
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.47)

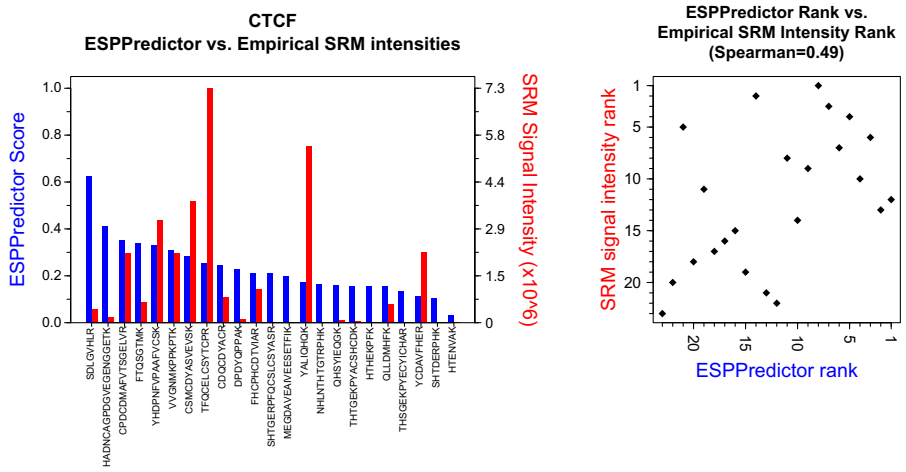


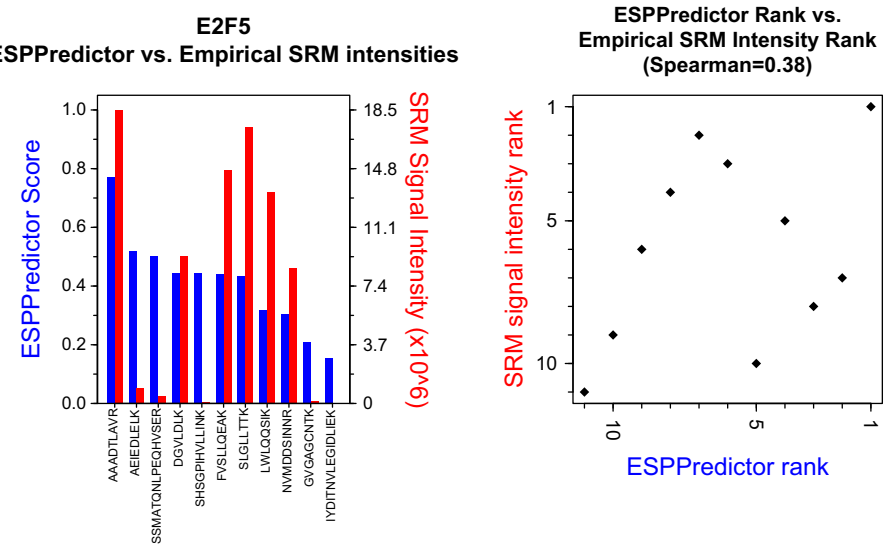
CLOCK
ESPPredictor vs. Empirical SRM intensities

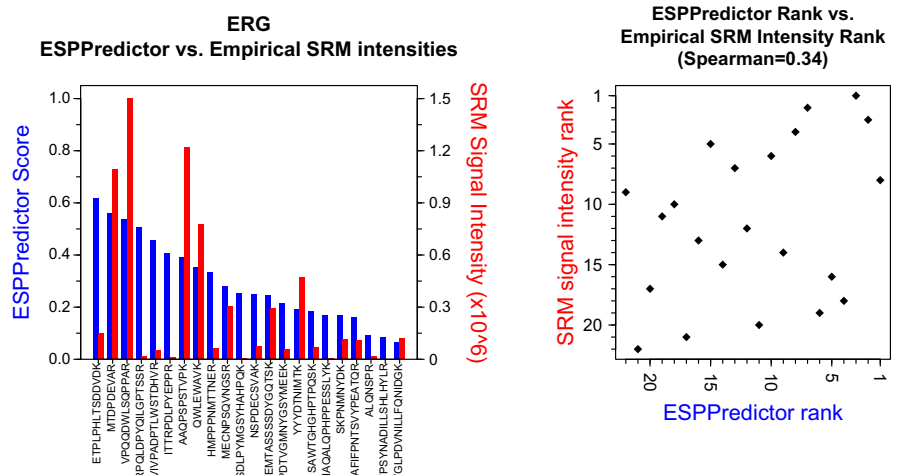


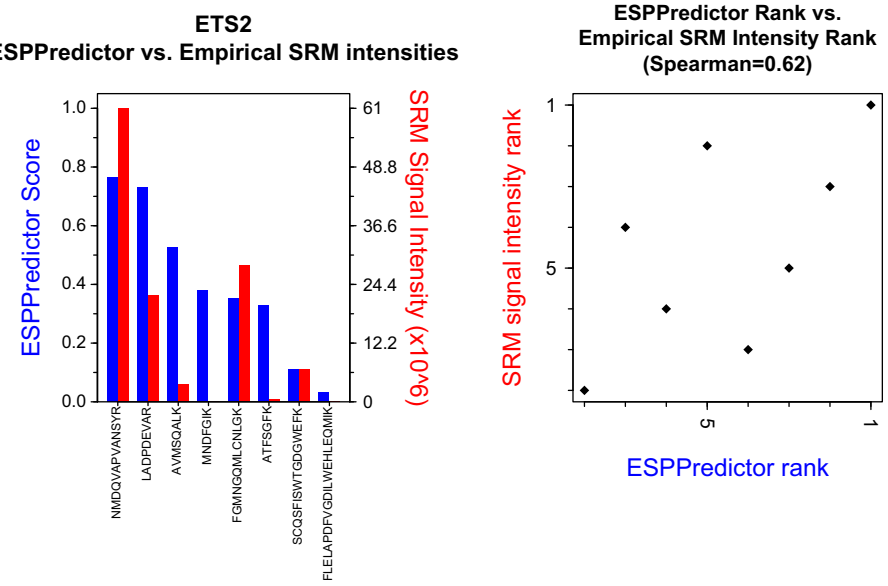
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.49)



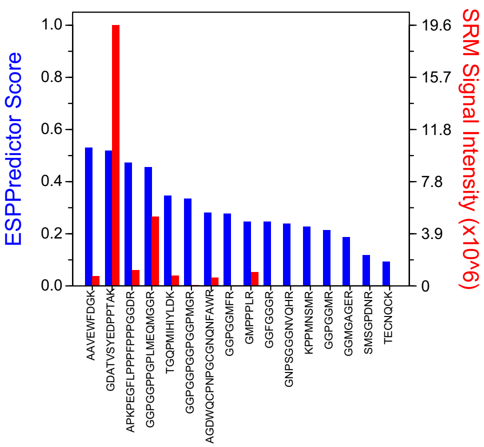

CTCF
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
SDLGVHLR
HADNCAGPDGVEGENGGETK
CPDCDMAPVTSGELVR
FTQSGTMK
YHDPNFVPAAFVCSK
VVGNMKPPKPTK
CSMCDYASVEVSK
TFQCELCSYTCPR
CDQCDYACR
DPDYQPPAK
FHCPHCDTVAR
SHTGERPFQCSLCSYASR
MEGDAVEAVEESETFIK
YALIQHQK
NHLNTHTGTRPHK
QHSVIEQGK
THTGEKPYACSHCDK
HTHEKPFK
QLLDMHFK
THSGEKPYECYICHAR
YCDAVFHER
SHTDERPHK
HTENVAK
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.49)
SRM signal intensity rank
ESPPredictor rank


E2F5
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
AAADTLAVR
AEIEDLELK
SSMATQNLPEQHVSER
DGVLDLK
SHSGPIHVLLINK
FVSLLQEAK
SLGLLTTK
LWLQQSIK
NVMDDSINNR
GVGAGGNTK
IYDITNVLEGIDLIEK
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.38)
SRM signal intensity rank
ESPPredictor rank

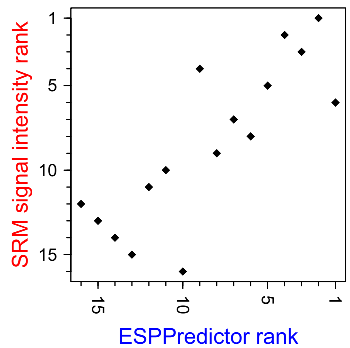

ERG
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
ETPLPHLTSDDVDK
MTDPDEVAR
VPQQDWLSQPPAR
TEDQRQLDPYQILGPTSSR
VIVPADPTLWSTDHVR
ITTRPDLPYEPPR
AAQPSPSTVPK
QWLEWAVK
HMPPPNMTTNER
MECNPSQVNGSR
YPSDLPYMGSYHAHPQK
NSPDECSVAK
TEMTASSSSDYGQTSK
MVGSPDTVGMNYGSYMEEK
YYYDTNIMTK
SAWTGHGHPTPQSK
FDFHGIAQALQPHPPESSLYK
SKPNMNYDK
NTGGAAFIFPNTSVYPEATQR
ALQNSPR
LTPSYNADILLSHLHYLR
EYGLPDVNILLFQNIDGK
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.34)
SRM signal intensity rank
ESPPredictor rank


ETS2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
NMDQVAPVANSYR
LADPDEVAR
AVMSQALK
MNDFGIK
FGMNGQMLCNLGK
ATFSGFK
SCQSFISWTGDGWEFK
FLELAPDFVGDILWEHLEQMIK
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.62)
SRM signal intensity rank
ESPPredictor rank

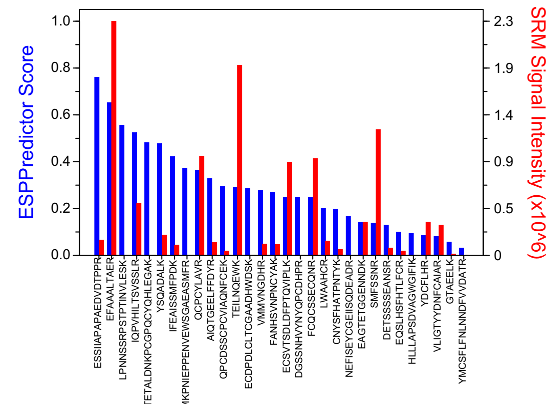
EWSR1
ESPPredictor vs. Empirical SRM intensities


ESPPredictor Score
SRM Signal Intensity (x10^6)
1.0
0.8
0.6
0.4
0.2
0.0
19.6
15.7
11.8
7.8
3.9
0
AAVEWFDGK
GDATVSYEDPPTAK
APKPEGFLPPPFPPPGDR
GGPGGPGPLMEQMGGR
TGQPMIHIYLDK
GGPGPGGPGGPMGR
AGDWQCPNPGCGNQNFAWR
GGPGGMFR
GMPPPLR
GGFGGGR
GNPSGGGNVQHR
KPPMNSMR
GGPGGMR
GGMGAGER
SMSGPDNR
TECNQCK

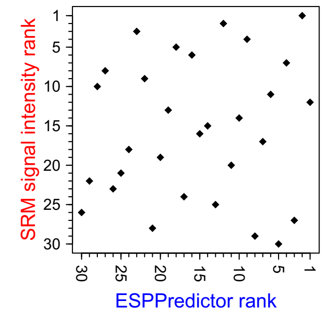
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.82)



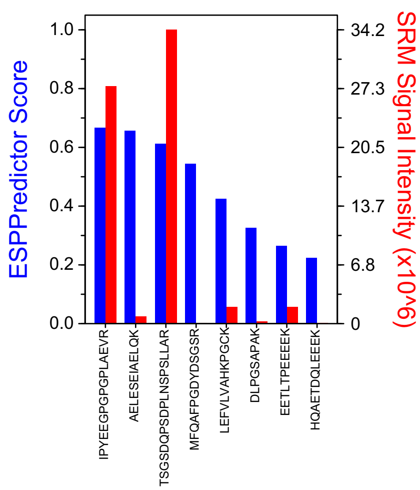
EZH2
ESPPredictor vs. Empirical SRM intensities



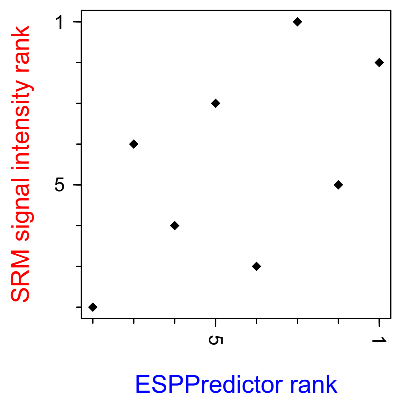
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.099)



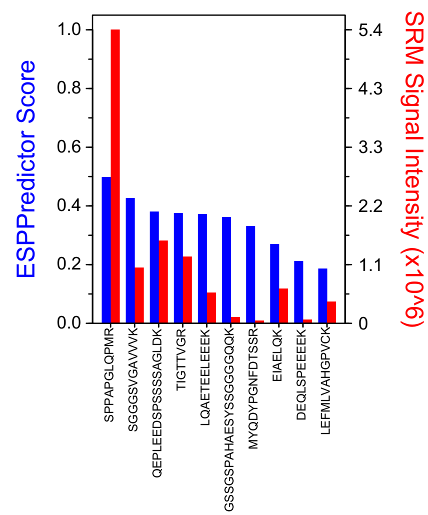
FOSB
ESPPredictor vs. Empirical SRM intensities



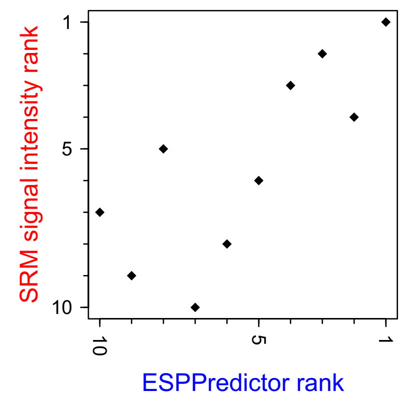
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.57)

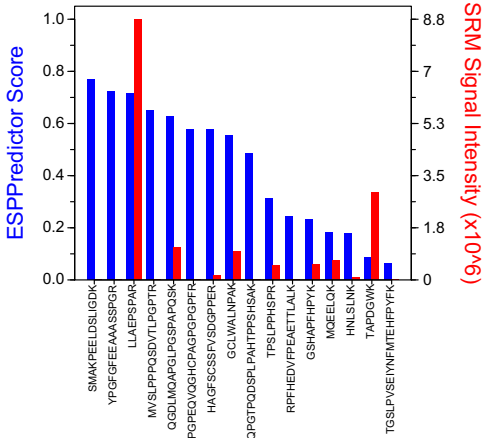


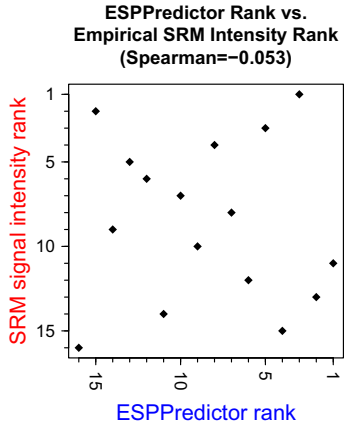
FOSL2
ESPPredictor vs. Empirical SRM intensities

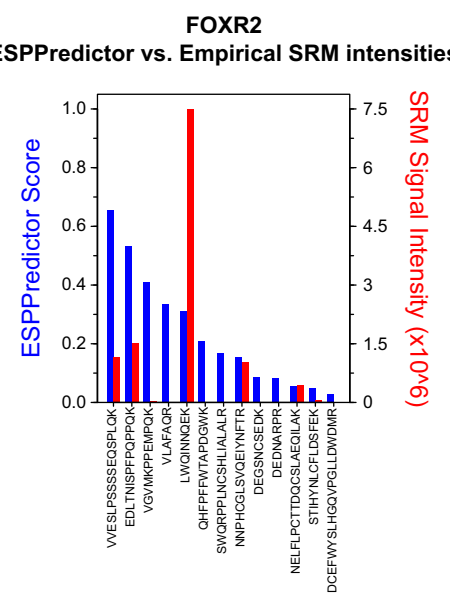


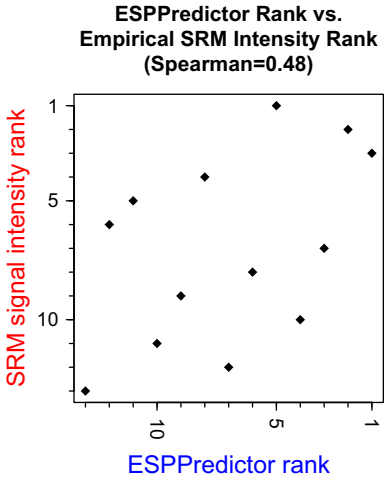
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.77)

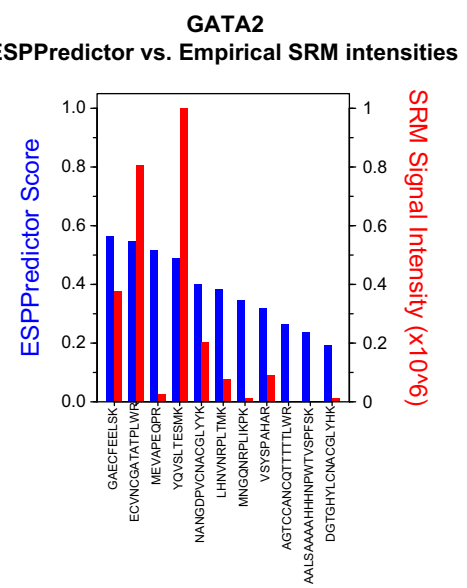


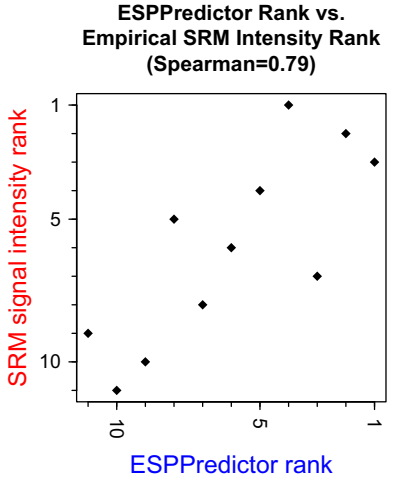

FOXN1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
SMAKPEELDSLIGDK
YPGFGFEEAAASSPGR
LLAEPSPAR
MVSLPPPQSDVTLPGPTR
QGDLMQAPGLPGSPAPQSK
IASPGPEQVQGHCPAGPGGPFR
HAGFSCSSRV/SDGPPER
GCLWALNPAK
AQPGTPQDSPLPAHTPPSHSAK
TPSLPPHSPR
RPPHEDVFPEAETTLALK
GSHAPFHPYK
MQEELQK
HNLSLNK
TAPDGWK
TGSLPVSEIYNFMTEHFPYFK

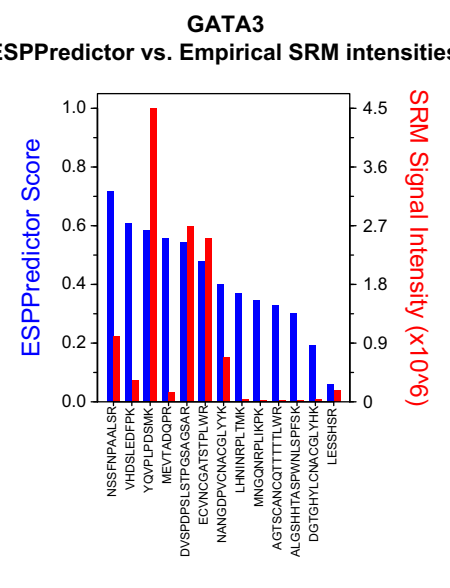

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=−0.053)
SRM signal intensity rank
ESPPredictor rank

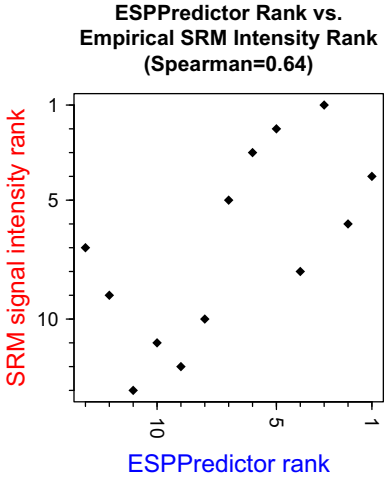

FOXR2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
VVESLPSSSSEQSPLQK
EDLTNISPFPQPPQK
VGVMKPPEMPQK
VLAFAQR
LWQINNQEK
QHFPFFWTAPDGWK
SWQRPPLNCSHLIALALR
NNPHCGLSVQEIYNFTR
DEGSNCSEDK
DEDNARPR
NELFLPCTTDQCSLAEQILAK
STIHYNLCFLDSFEK
DCEFWYSLHGQVPGLLDWDMR

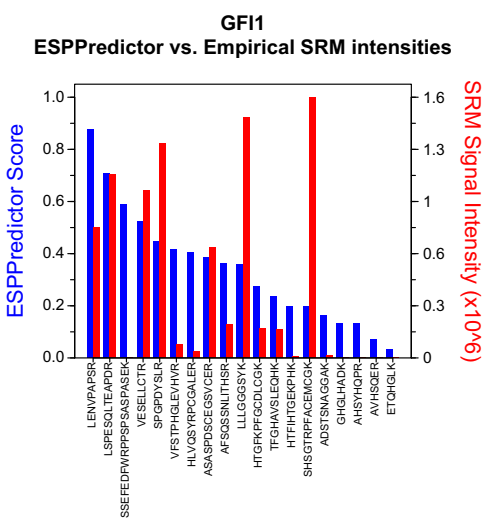

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.48)
SRM signal intensity rank
ESPPredictor rank

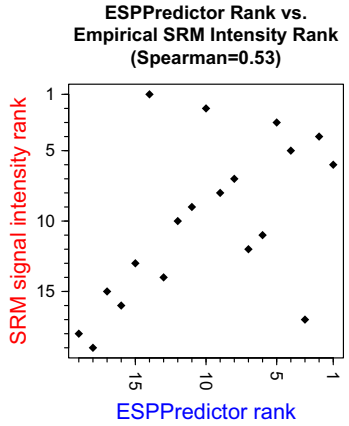

GATA2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
GAECFEELSK
ECVNCGATATPLWR
MEVAPEQPR
YQVSLTESMK
NANGDPVCNACGLYYK
LHNVNRPLTMK
MNGQNRPLIKPK
VSYSPAHAR
AGTCCANCQTTTTTLWR
AALSAAAAHHNPWTVSPFSK
DGTGHYLCNACGLYHK

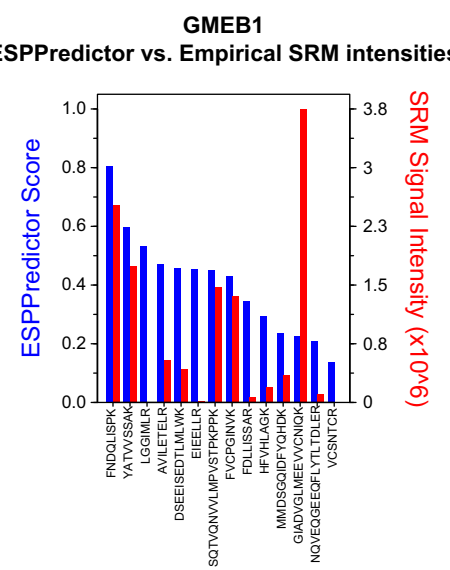

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.79)
SRM signal intensity rank
ESPPredictor rank

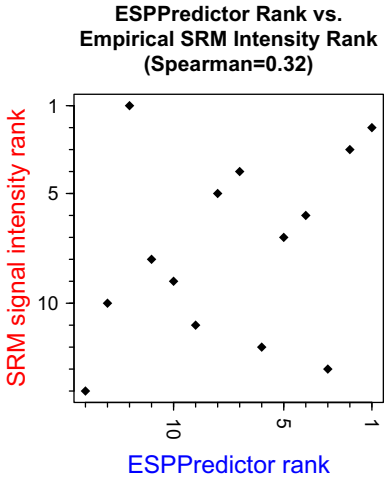

GATA3
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
NSSFNPAALSR
VHDSLEDFPK
YQVPLPDSMK
MEVTADQPR
DVSPDPSLSTPGSAGSAR
ECVNCGATSTPLWR
NANGDPVCNACGLYYK
LHNINRPLTMK
MNGQNRPLIKPK
AGTSCANCQTTTTTLWR
ALGSHITASPWNLSPFSK
DGTGHYLCNACGLYHK
LESSHSR

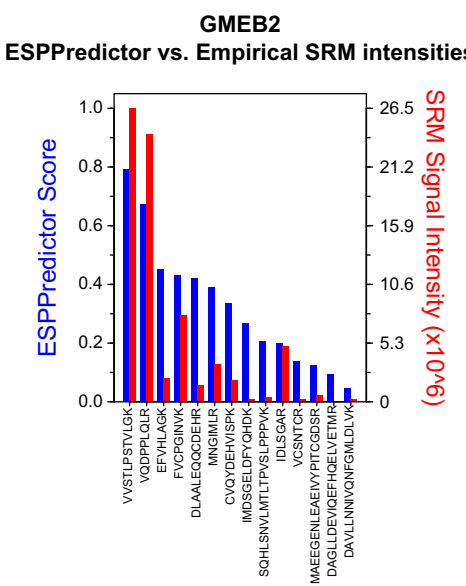

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.64)
SRM signal intensity rank
ESPPredictor rank

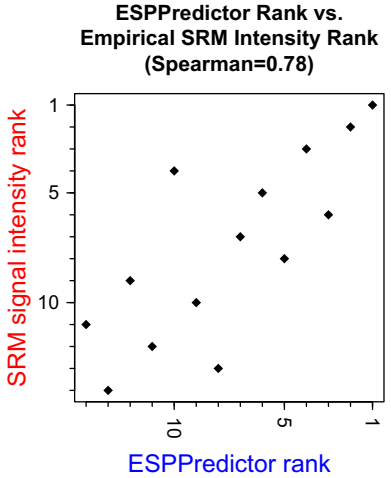

GFI1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
LENVPAPSR
LSPESQLTEAPDR
SSEFEDPWRPPSPSASPASEK
VESELLCTR
SPGPDYSLR
VFSTPHGLEVHVR
HLVQSYRPCGALER
ASASPDSCEGSVCER
AFSQSSNLITHSR
LLLGGGSYK
HTGFKPFSCDLCGK
TFGHAVSLEQHK
HTFIHTGEKPHK
SHSGTRPFACMCGK
ADSTSNAGGAK
GHGLHADK
AHSYHQPR
AVHSQER
ETQHGLK

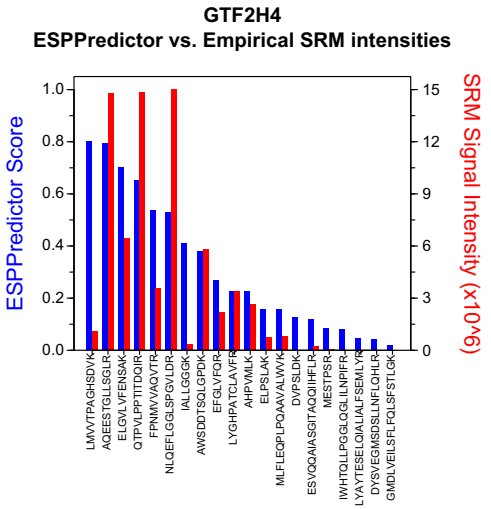

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.53)
SRM signal intensity rank
ESPPredictor rank

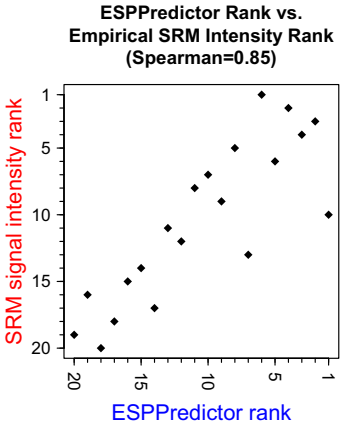

GMEB1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
FNDQLISPK
YATVVSSAK
LGGIMLR
AVILETELR
DSEEISEDTLMLWK
EIEELLR
SQTVQNVVLMPVSTPKPPK
FVCPGINVK
FDLLISSAR
HFVHLAGK
MMDSGQIDFYQHDK
GIADVGLMEEVVCNIQK
NQVEQGEEQFLYTLTDLER
VCSNTCR


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.32)
SRM signal intensity rank
ESPPredictor rank

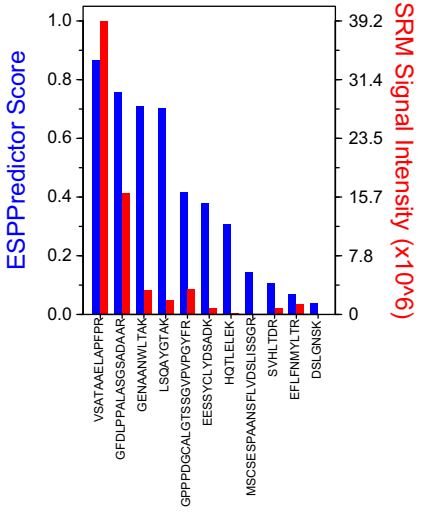

GMEB2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
VVSTLPSTVLGK
VQDPPLQLR
EFVHLAGK
FVCPGINVK
DLAALEQQCDEHR
MNGIMLR
CVQYDEHVISPK
IMDSGELDFYQHDK
SQHLSNVLMTLTPVSLPPPVK
IDLSGAR
VCSNTCR
MAEEGENLEAEIVYPITCGDSR
DAGLLDEVIQEFHQELVETMR
DAVLLNNIVQNFGMLDLVK


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.78)
SRM signal intensity rank
ESPPredictor rank

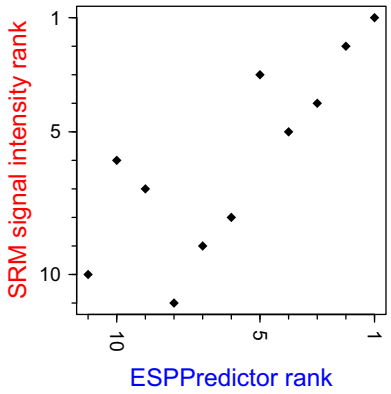

GTF2H4
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
LMVVTPAGHSDVK
AQEESTGLLSGLR
ELGVLVFENSAK
QTPVLPPTITDQIR
FPNMVVAQVTR
NLQEFLGGLSPGVLDR
IALLGGGK
AWSDDTSQLGPDK
EFGLVFQR
LYGHPATCLAVFR
AHPVMLK
ELPSLAK
MLFLEQPLPQAAVALWVK
DVPSLDK
ESVQQAIASGITAQQIHFLR
MESTPSR
IWHTQLLPGGLQGLILNPIFR
LYAYTESELQIALIALFSEMLYR
DYSVEGMSDSLLNFLQHLR
GMDLVEILSRLFQLSFSTLGK


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.85)
SRM signal intensity rank
ESPPredictor rank

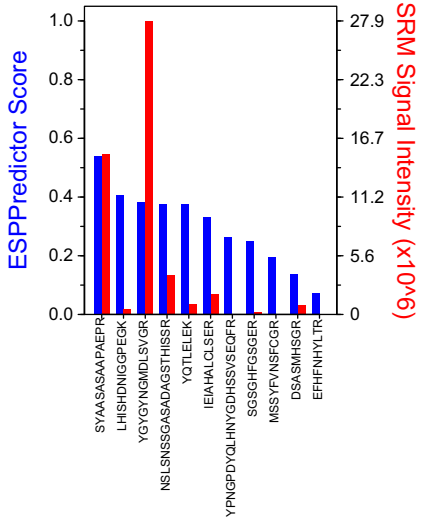
HOXA10
ESPPredictor vs. Empirical SRM intensities



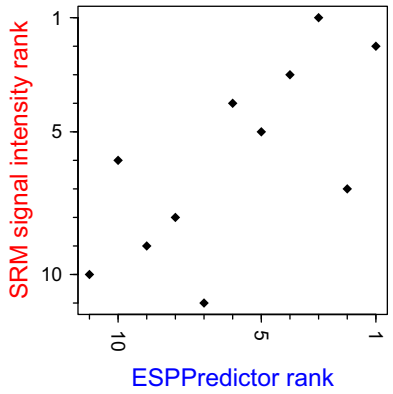
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.8)



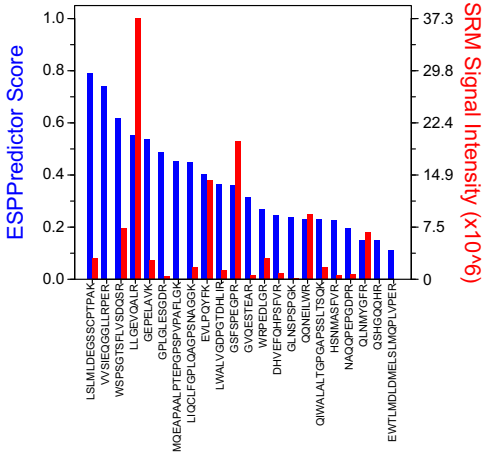
HOXA5
ESPPredictor vs. Empirical SRM intensities



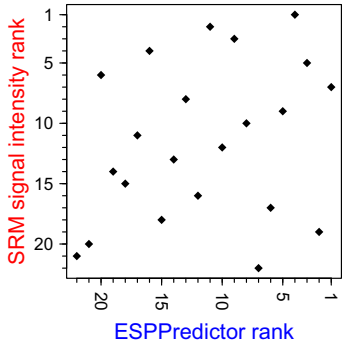
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.69)



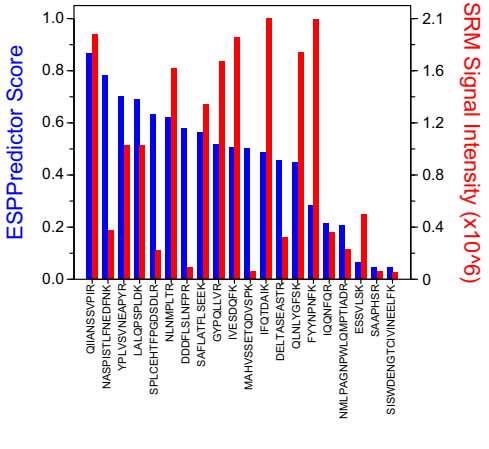
HSF4
ESPPredictor vs. Empirical SRM intensities



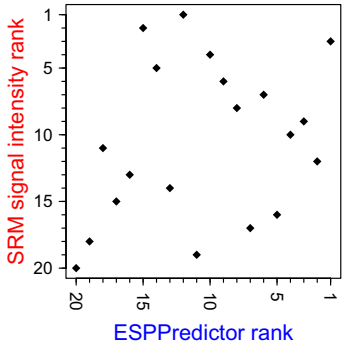
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.27)



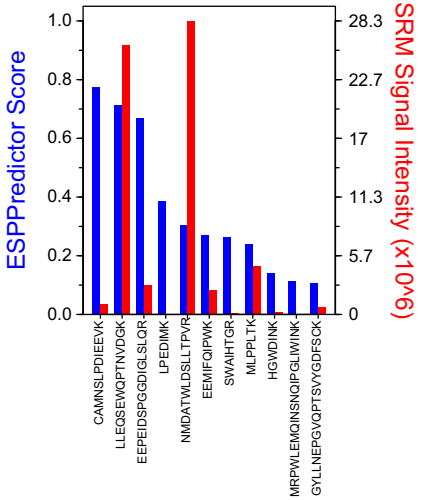
HSFY2
ESPPredictor vs. Empirical SRM intensities



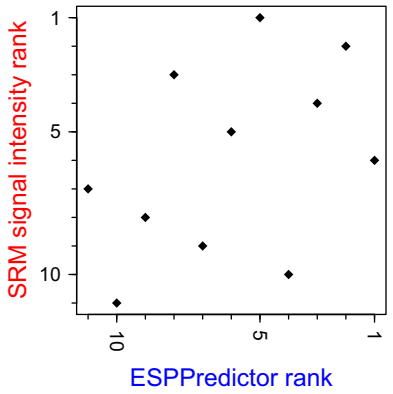
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.29)



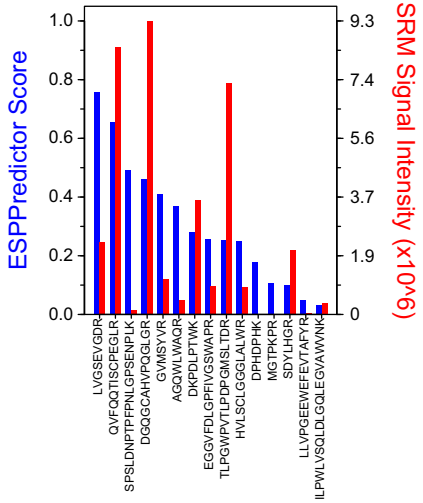
IRF1
ESPPredictor vs. Empirical SRM intensities



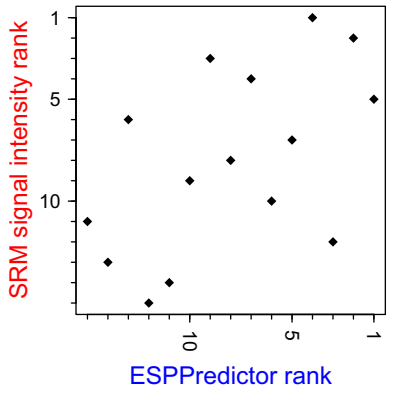
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.43)



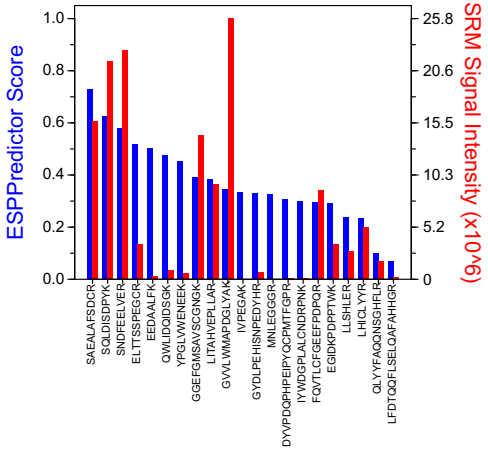
IRF3
ESPPredictor vs. Empirical SRM intensities



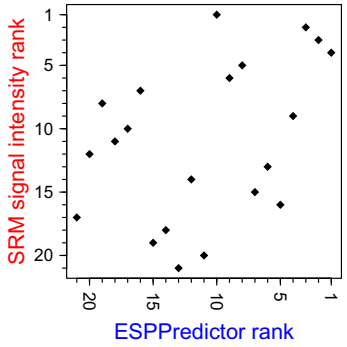
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.54)



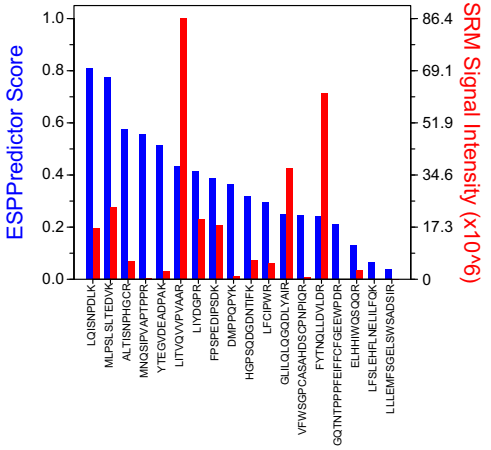
IRF4
ESPPredictor vs. Empirical SRM intensities



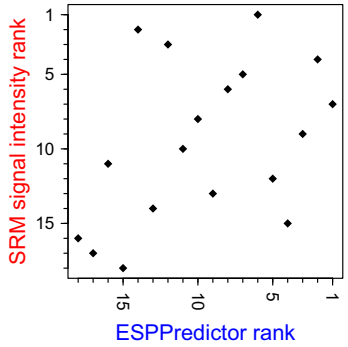
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.4)



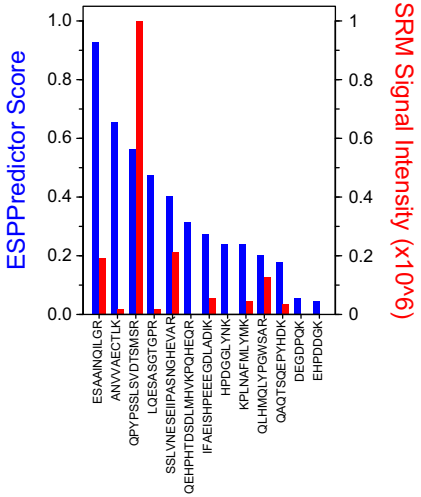
IRF5
ESPPredictor vs. Empirical SRM intensities



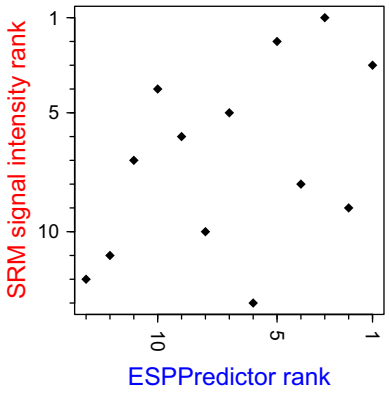
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.42)



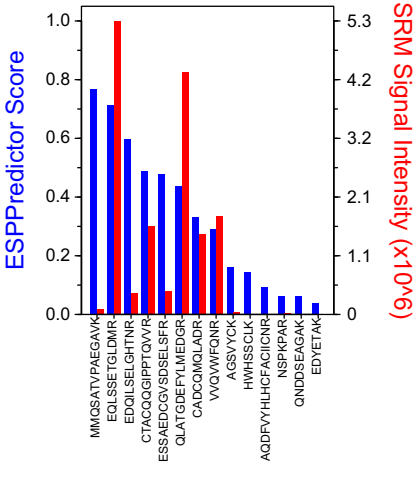
LEF1
ESPPredictor vs. Empirical SRM intensities



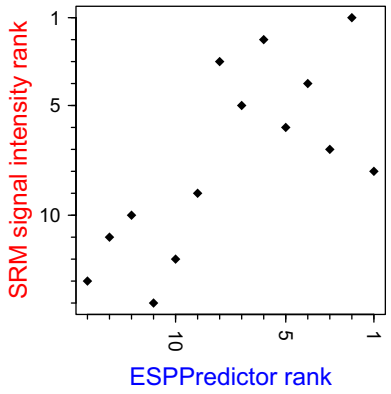
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.45)



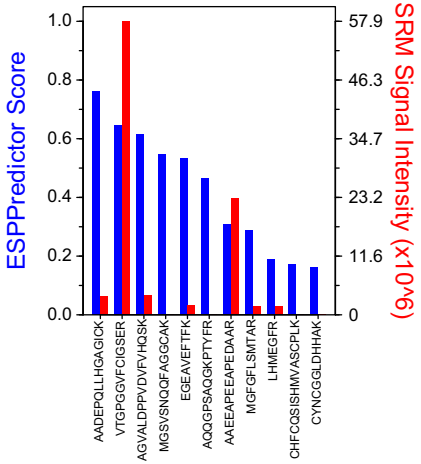
LHX4
ESPPredictor vs. Empirical SRM intensities



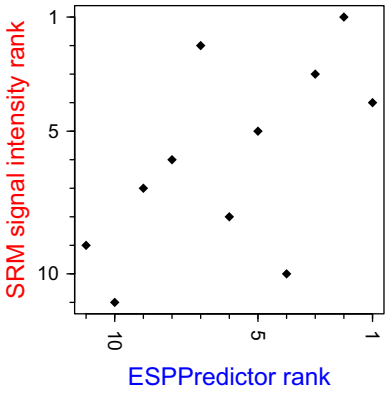
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.71)



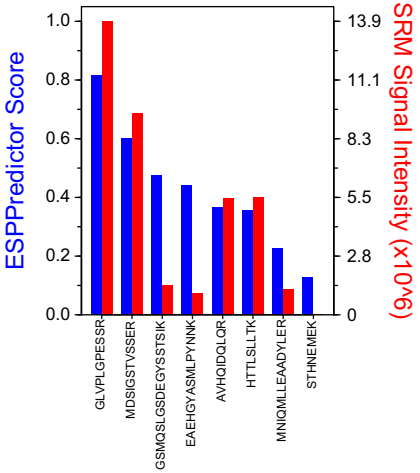
LIN28
ESPPredictor vs. Empirical SRM intensities



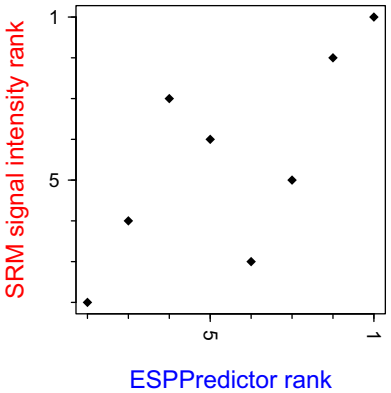
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.6)



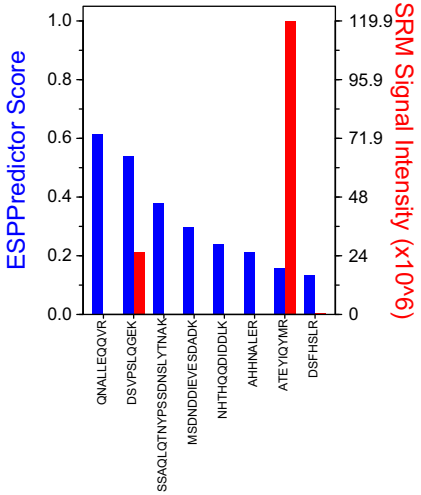
MAD
ESPPredictor vs. Empirical SRM intensities



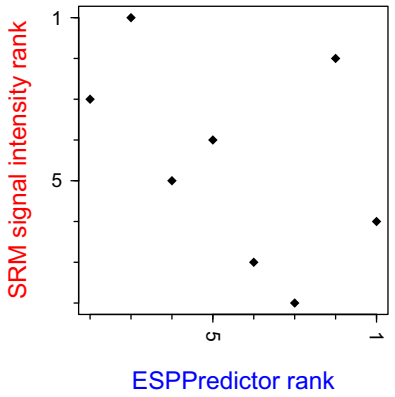
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.71)



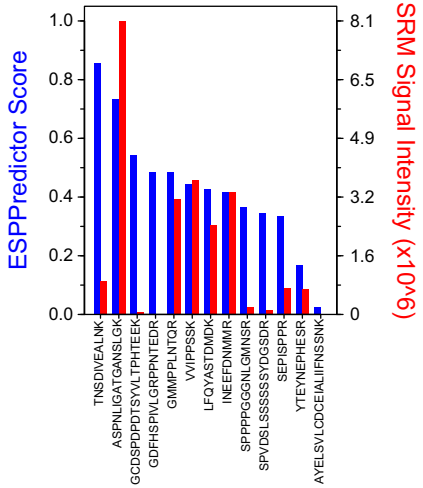
MAX
ESPPredictor vs. Empirical SRM intensities



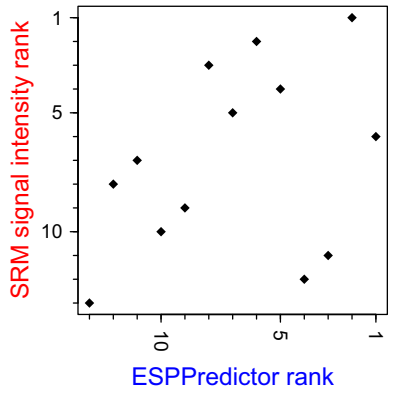
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=−0.45)



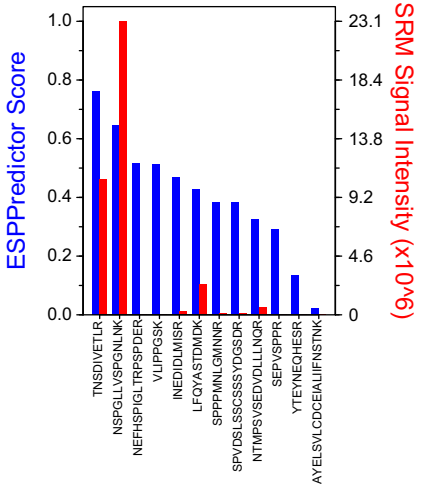
MEF2A
ESPPredictor vs. Empirical SRM intensities



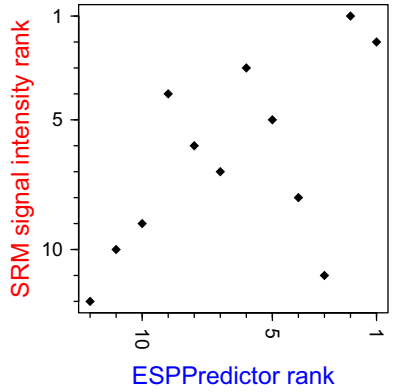
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.36)



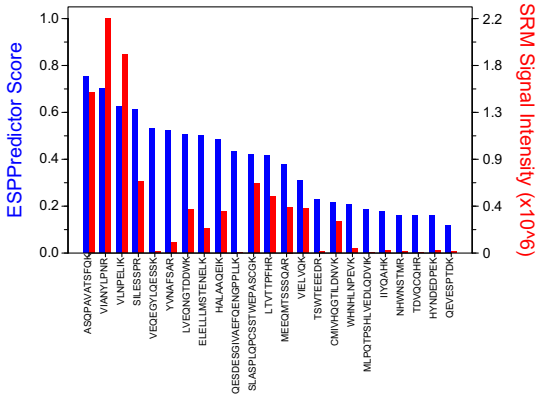
MEF2C
ESPPredictor vs. Empirical SRM intensities



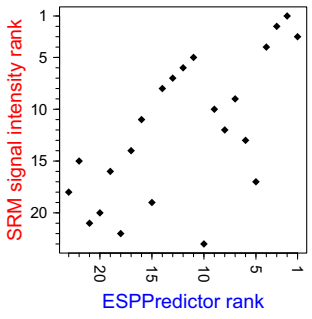
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.57)

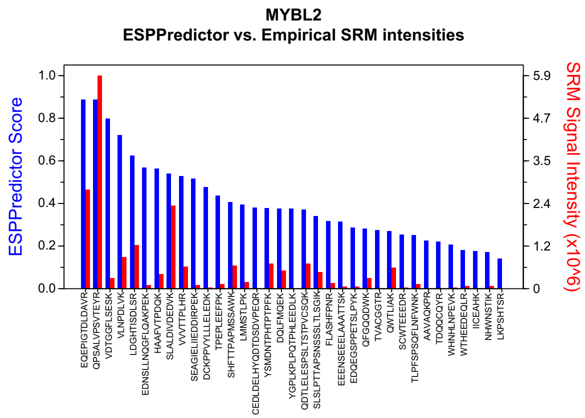


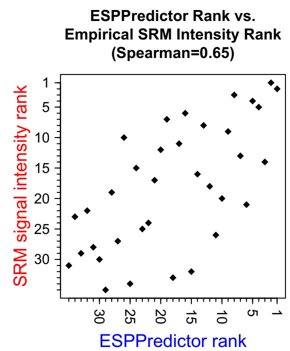
MYB
ESPPredictor vs. Empirical SRM intensities

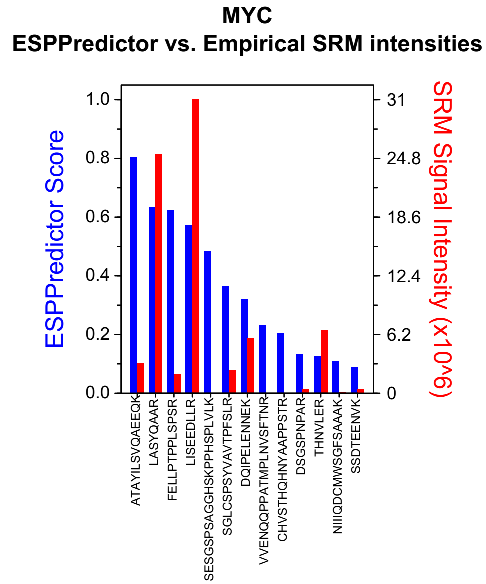


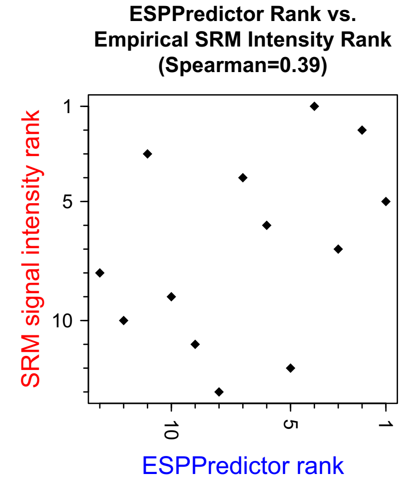
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.66)

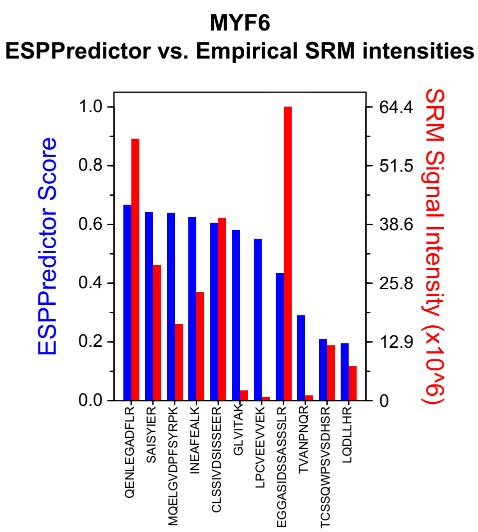


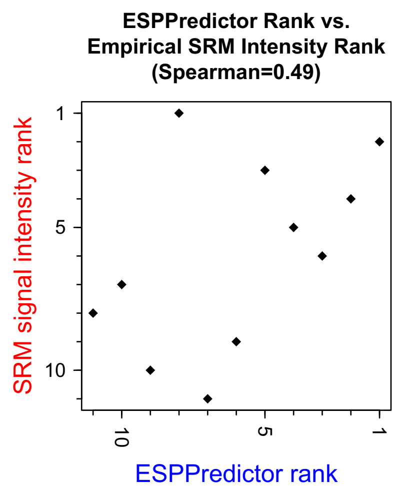

MYBL2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)

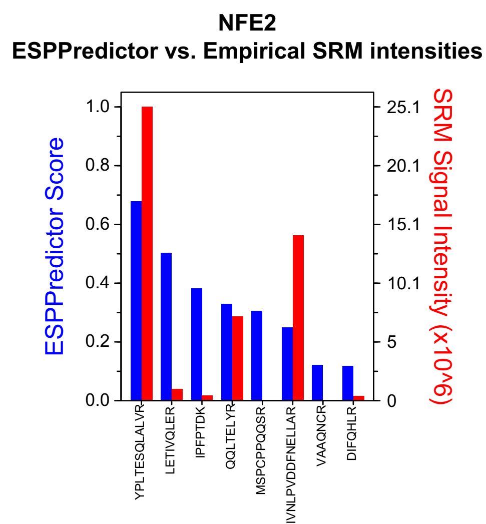

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.65)
SRM signal intensity rank
ESPPredictor rank

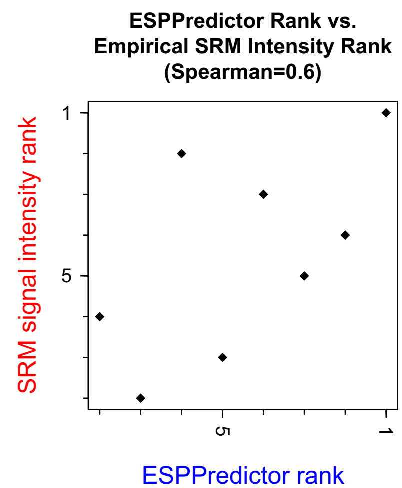

MYC
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
ATAYILSVQAEEQK
LASYQAAR
FELLPTPPLSPSR
LISEEDLLR
SESGSPSAGGHSKPPHSPLVLK
SGLCSPSYVAVTPFSLR
DQIPELENNEK
VVENQPPPATMPLNVSFTNR
CHVSTHQHNYAAPPSTR
DSGSPNPAR
THNVLER
NIIIQDCMWSGFSAAAK
SSDTEENVK


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.39)
SRM signal intensity rank
ESPPredictor rank

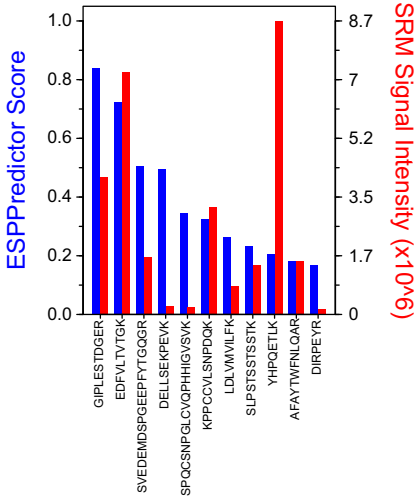

MYF6
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
QENLEGADFLR
SAISYIER
MQELGVDPFSYRPK
INEAFEALK
CLSSIVDSISSEER
GLVITAK
LPCVEEVVEK
EGGASIDSSASSSLR
TVANPNQR
TCSSQWPSVSDHSR
LQDLLHR


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.49)
SRM signal intensity rank
ESPPredictor rank

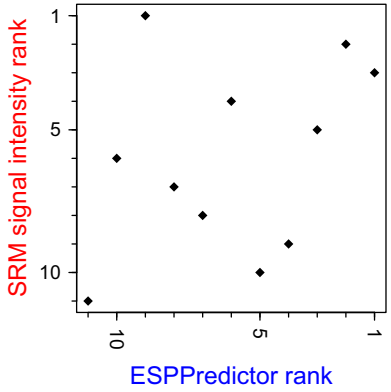

NFE2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
YPLTESQLALVR
LETIVQLER
IPFPTDK
QQLTELYR
MSPCPPQQSR
IVNLPVDDFNELLAR
VAAQNCR
DIFQHLR


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.6)
SRM signal intensity rank
ESPPredictor rank

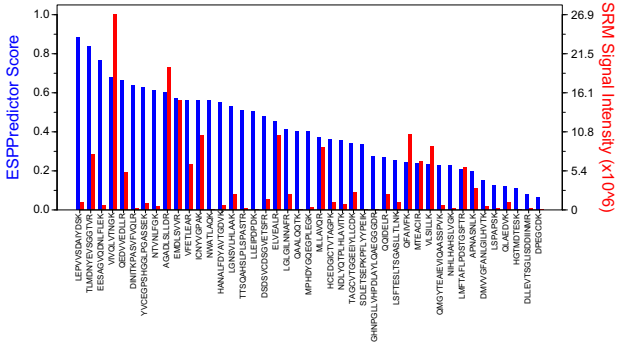
NFIA
ESPPredictor vs. Empirical SRM intensities



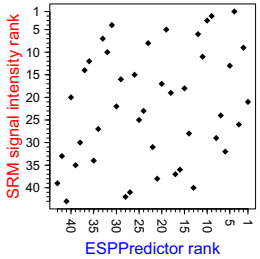
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.35)



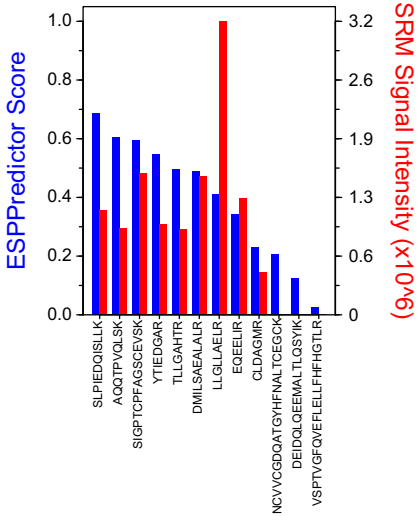
NFKB1
ESPPredictor vs. Empirical SRM intensities



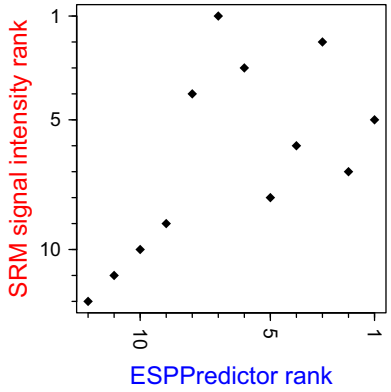
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.28)



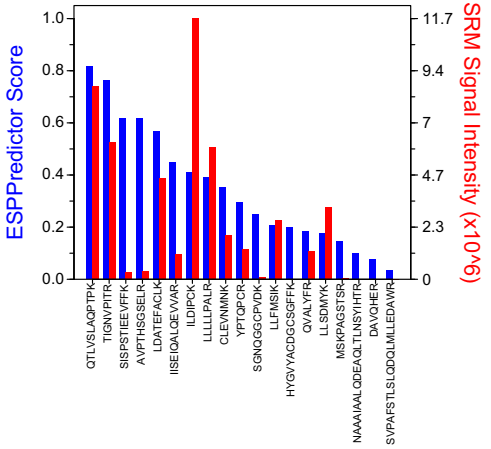
NR1I3
ESPPredictor vs. Empirical SRM intensities



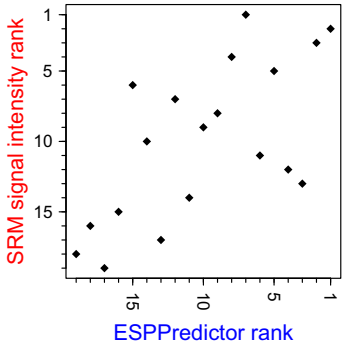
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.59)

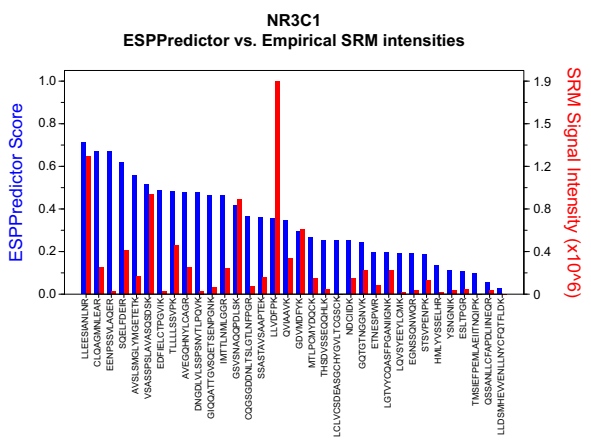


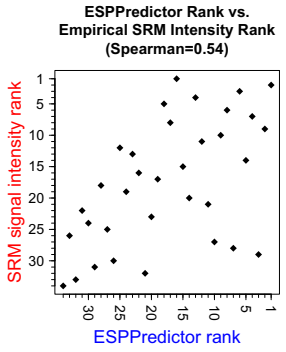
NR2E1
ESPPredictor vs. Empirical SRM intensities

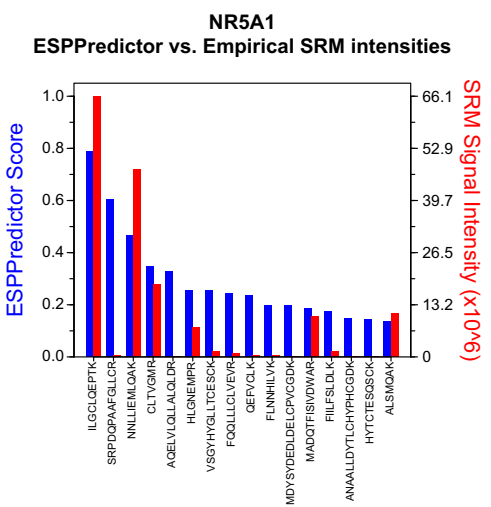


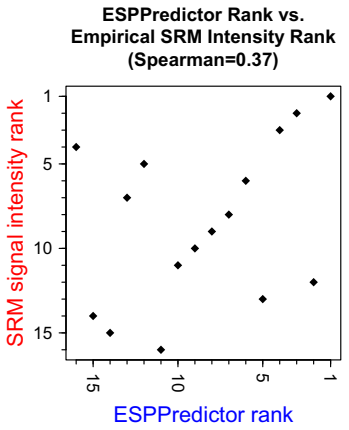
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.65)

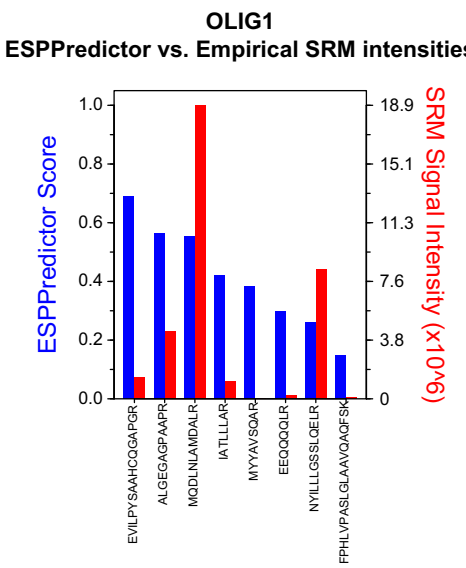


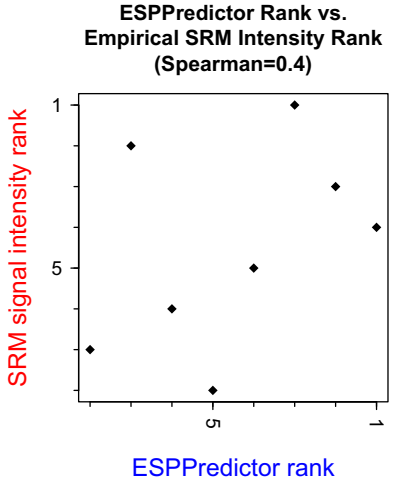

NR3C1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)

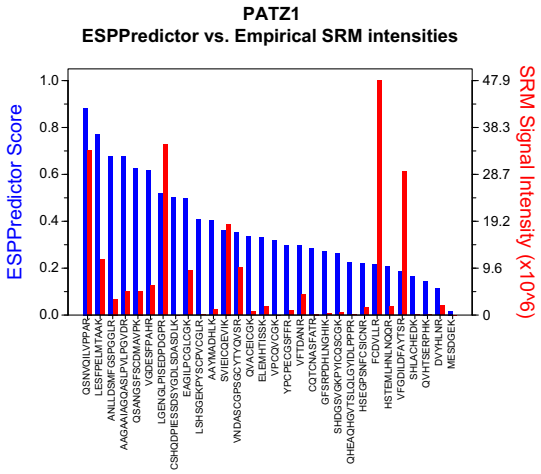

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.54)
SRM signal intensity rank
ESPPredictor rank

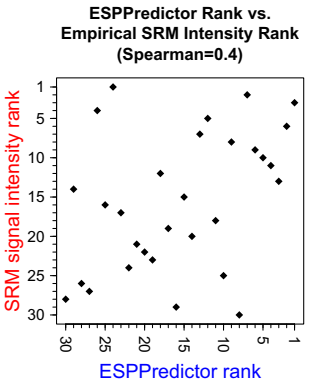

NR5A1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.37)
SRM signal intensity rank
ESPPredictor rank

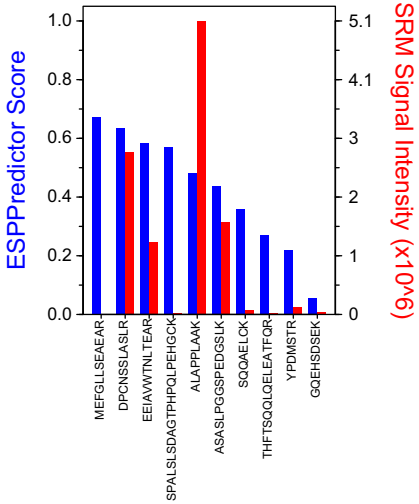

OLIG1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.4)
SRM signal intensity rank
ESPPredictor rank

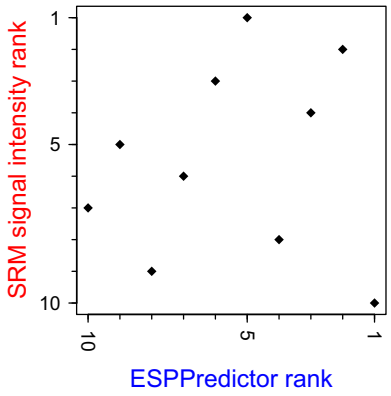

PATZ1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.4)
SRM signal intensity rank
ESPPredictor rank

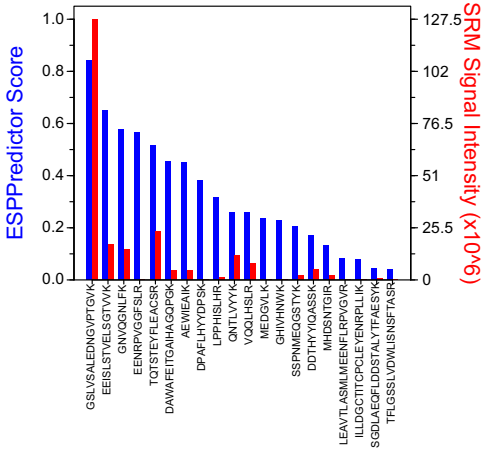
PITX3
ESPPredictor vs. Empirical SRM intensities



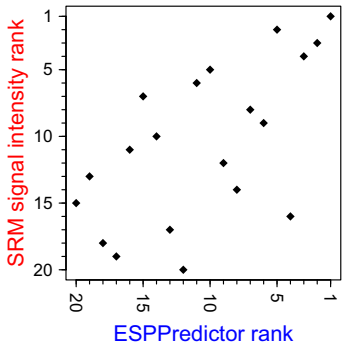
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.091)



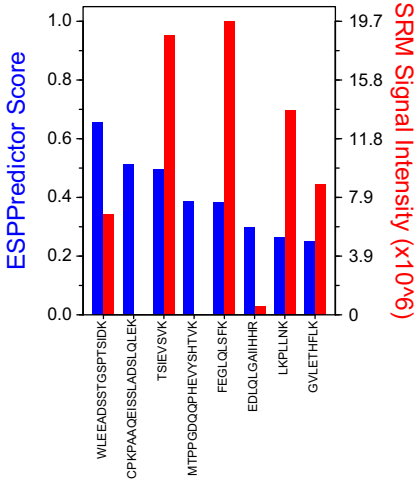
PLEK2
ESPPredictor vs. Empirical SRM intensities



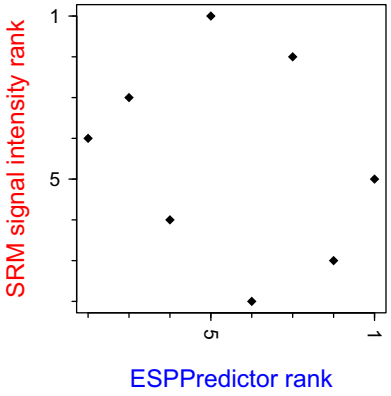
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.62)



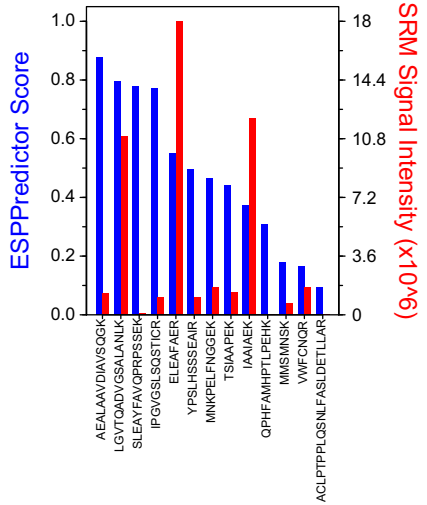
POU3F4
ESPPredictor vs. Empirical SRM intensities



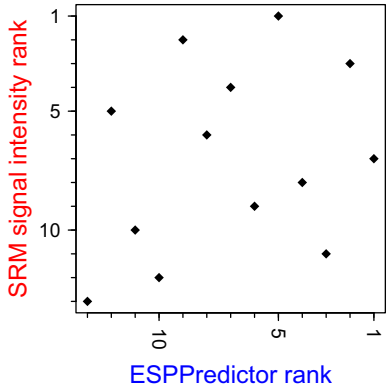
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=−0.26)



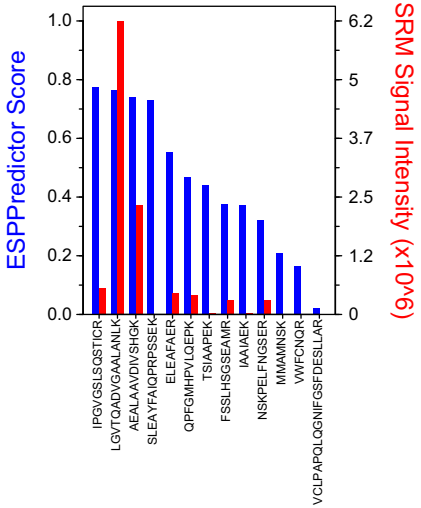
POU4F1
ESPPredictor vs. Empirical SRM intensities



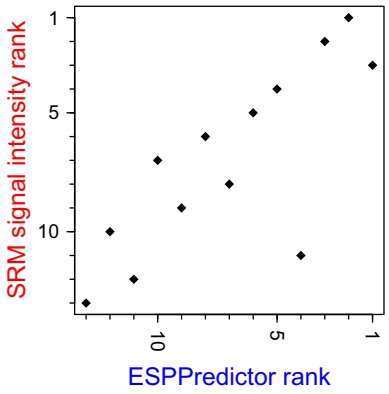
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.29)



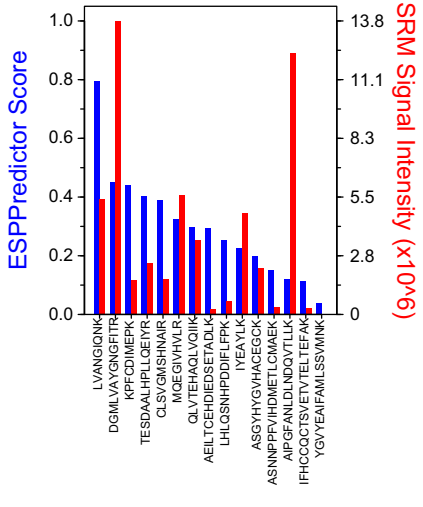
POU4F3
ESPPredictor vs. Empirical SRM intensities



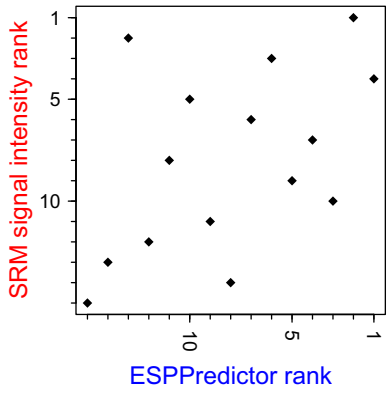
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.79)



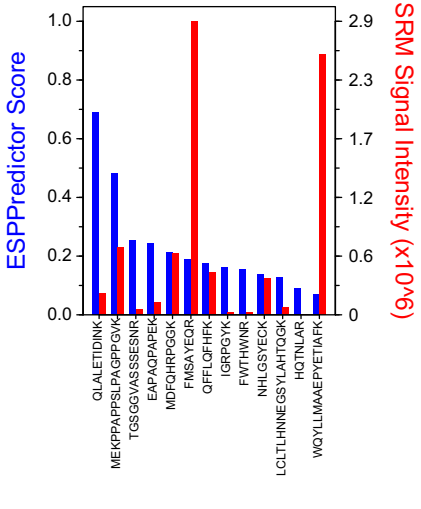
PPARA
ESPPredictor vs. Empirical SRM intensities



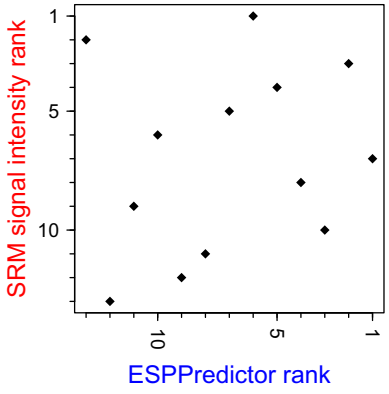
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.48)



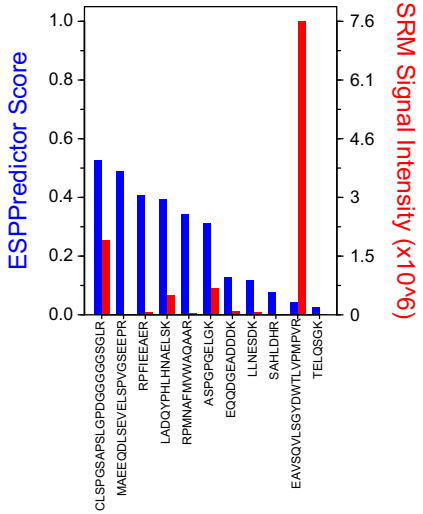
SF3A2
ESPPredictor vs. Empirical SRM intensities



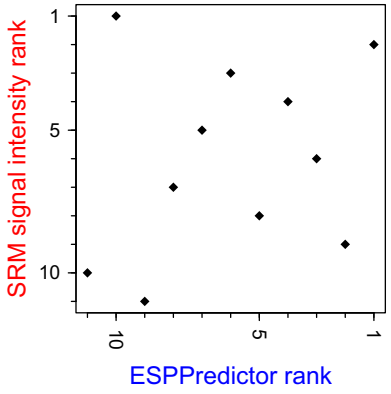
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.2)

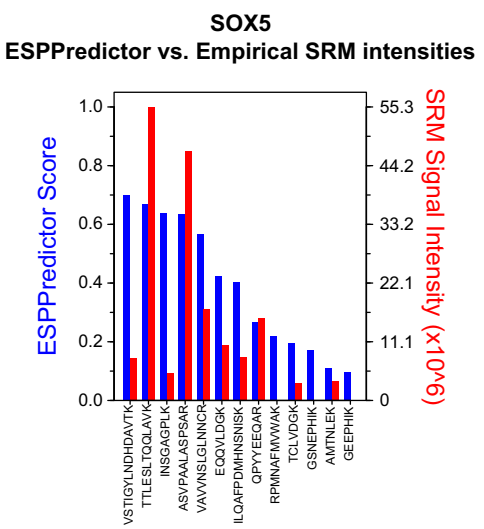


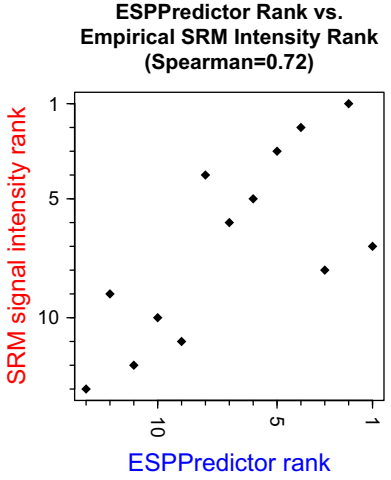
SOX10
ESPPredictor vs. Empirical SRM intensities

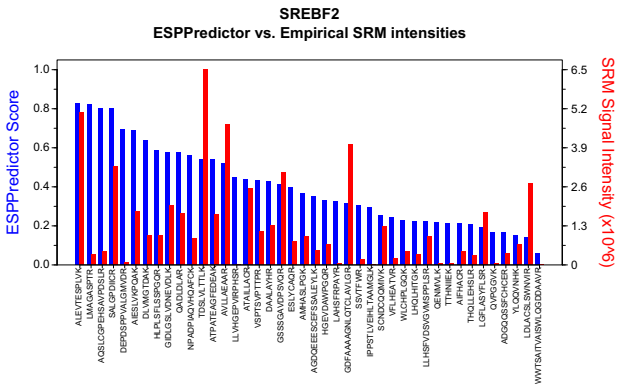


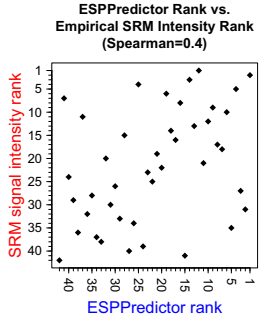
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.24)

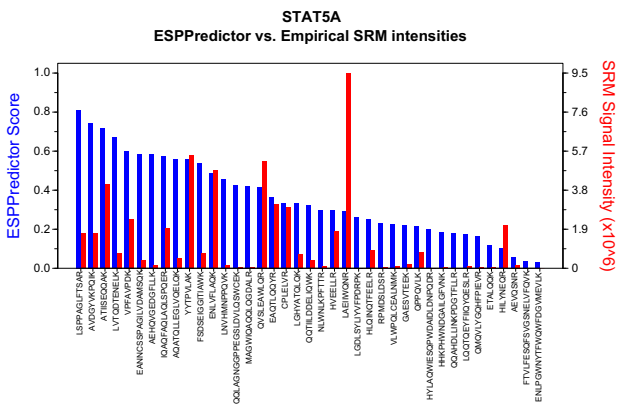


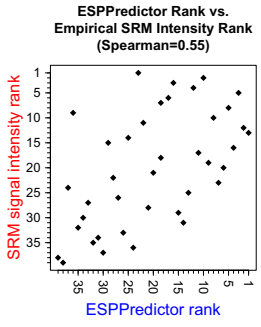

SOX5
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
VSTIGYLNDHDAVTK
TTLESLTQQLAVK
INSGAGPLK
ASVPAALASPSAR
VAVVNSLGLLNNCR
EQQVLDGK
ILQAFPDMHNSNISK
QPYYEEQAR
RPMNAFMVWAK
TCLVDGK
GSNEPHIK
AMTNLEK
GEEPHIK

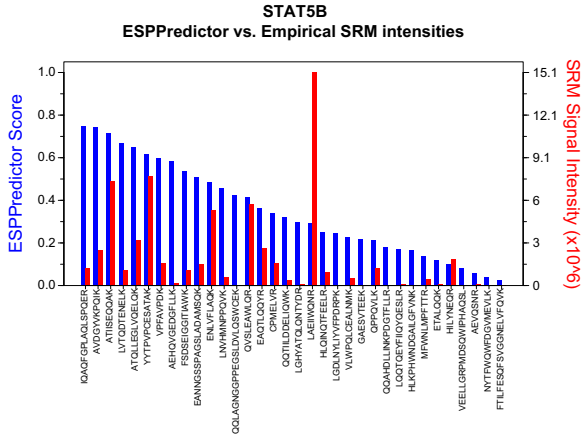

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.72)
SRM signal intensity rank
ESPPredictor rank

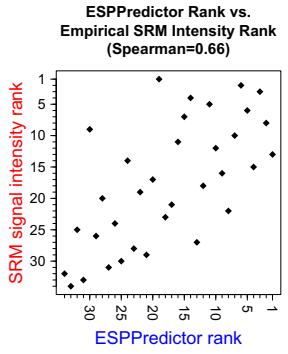

SREBF2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.4)
SRM signal intensity rank
ESPPredictor rank

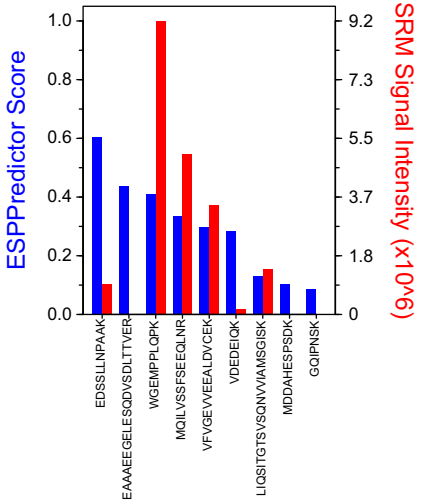

STAT5A
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.55)
SRM signal intensity rank
ESPPredictor rank

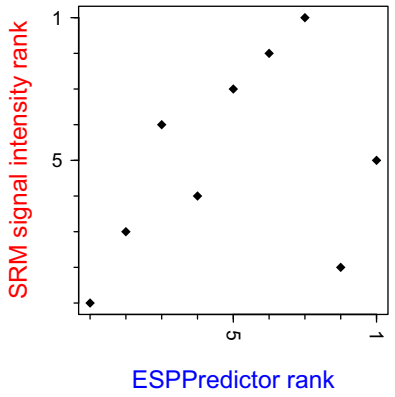

STAT5B
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.66)
SRM signal intensity rank
ESPPredictor rank

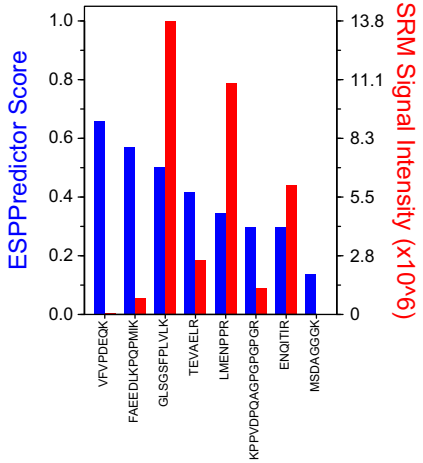
TAF11
ESPPredictor vs. Empirical SRM intensities



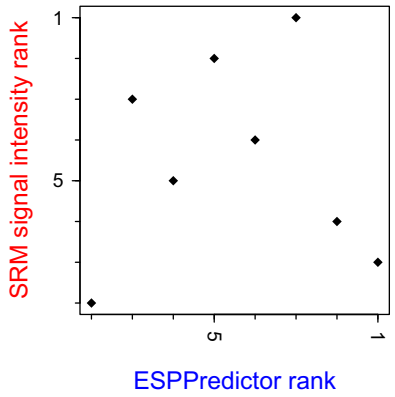
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.38)



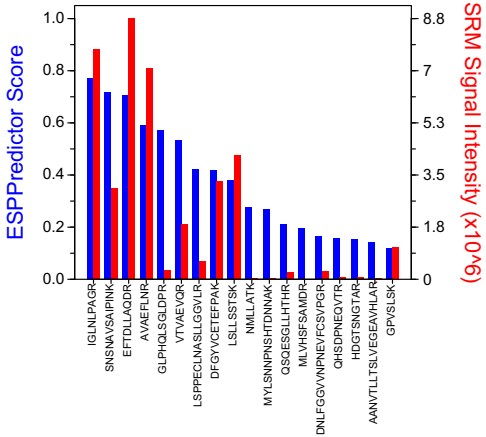
TEF
ESPPredictor vs. Empirical SRM intensities



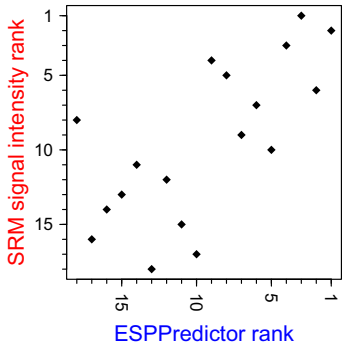
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.024)



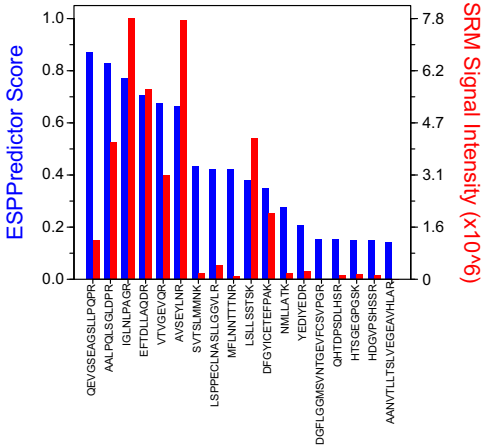
TFAP2A
ESPPredictor vs. Empirical SRM intensities



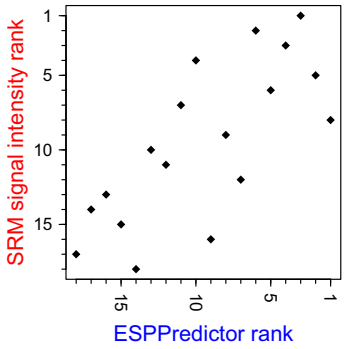
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.7)

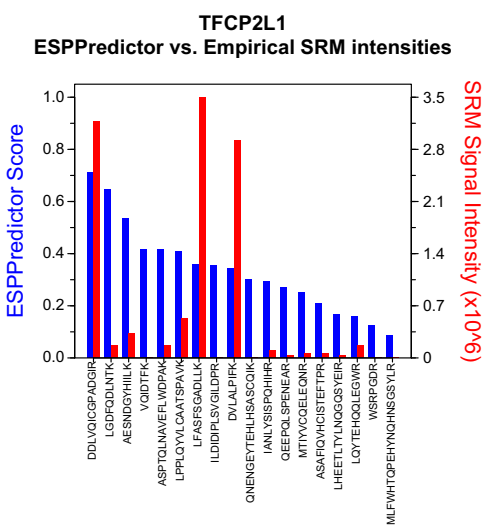


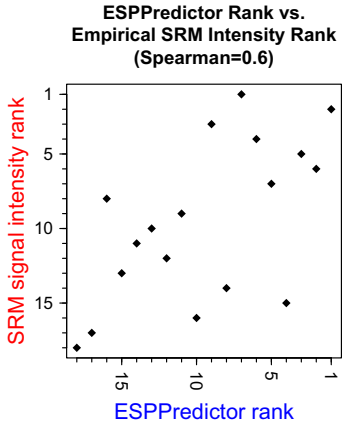
TFAP2B
ESPPredictor vs. Empirical SRM intensities

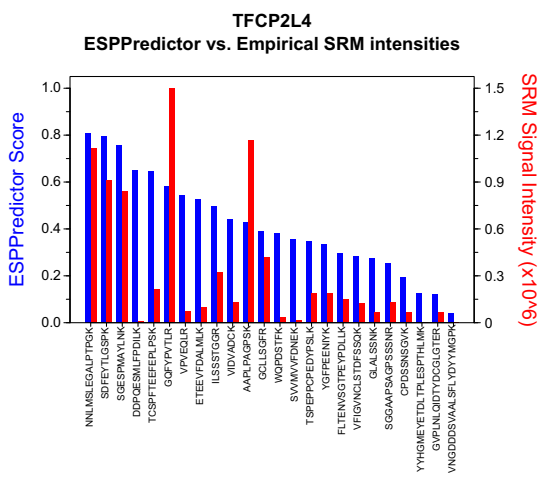


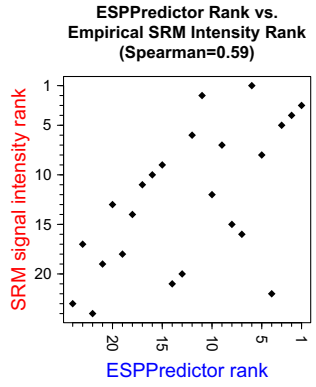
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.74)

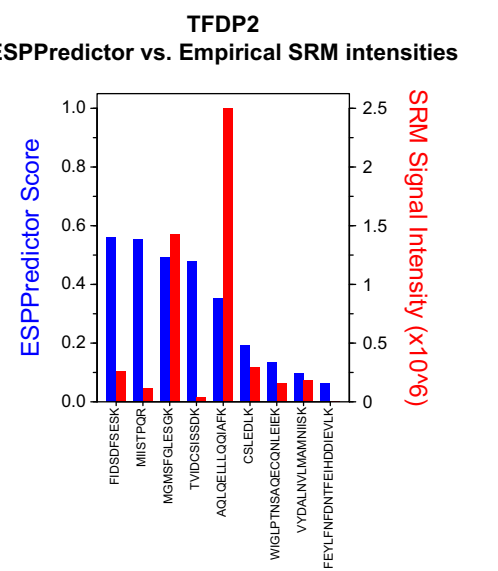


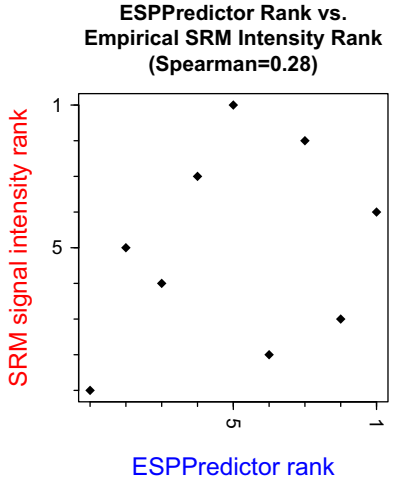

TFCP2L1
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
DDLVQICGPADGIR
LGDFQDLNTK
AESNDGYHILK
VQIDTFK
ASPTQLNAVEFLWDPAK
LPPLQYYLCAATSPAVK
LFASFSGADLLK
ILDIDIPLSVGILDPR
DVLALPIFK
QNENGEYTEHLHSASCQIK
IANLYSISPQHIHR
QEEPQLSPENEAR
MTIYVCQELEQNR
ASAFIQVHCISTEFTPR
LHEETLTYLNQGQSYEIR
LQYTEHQQLEGWR
WSRPGDR
MLFWHTQPEHYNQHNSGSYLR

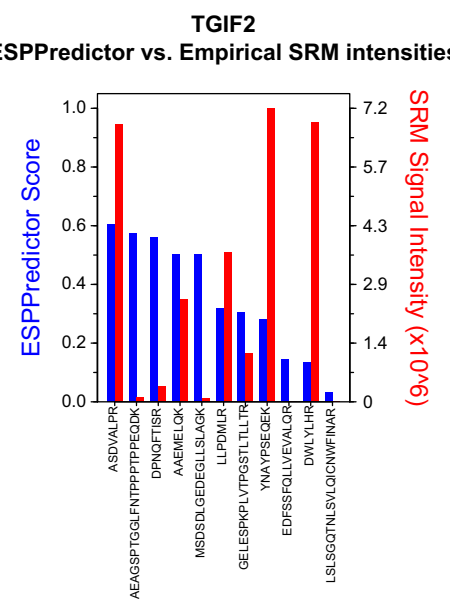

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.6)
SRM signal intensity rank
ESPPredictor rank

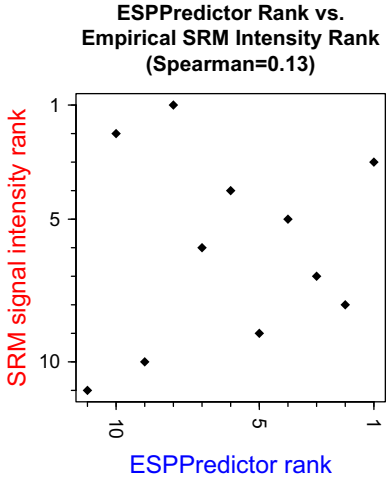

TFCP2L4
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
NNLMSLEGALPTPGK
SDFEYTLGSPK
SGESPMAYLMK
DDPQESMLFPDILK
TCSPFTEEFELPLPSK
GQFVPVTLR
VPVEQLR
ETEEVFDALMLK
ILSSSTGGR
VIDVADCK
AAPLPAGPSK
GCLLSGFR
WQPDSTFK
SVVMVFDNEK
TSPEPPCPEDYPSLK
YGFPEENIYK
FLTENVSGTPEYPDLLK
VFIGVNCLSTDFSSQK
GLALSSNK
SGGAAPSAGPSSSNR
CPDSSNSGVK
YYHGMEYETDLTPLESPTHLMK
GVPLNLQIDTYDCGLGTER
VNGDDDSVAALSFLYDYYMGPK


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.59)
SRM signal intensity rank
ESPPredictor rank

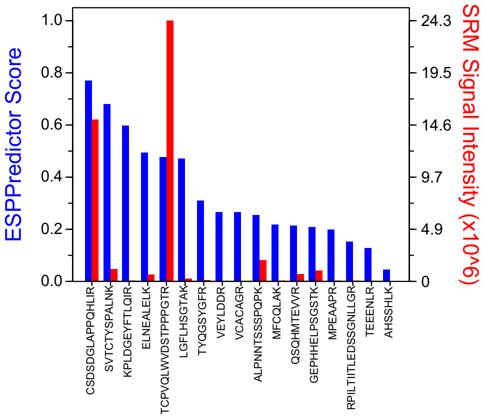

TFDP2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
FIDSDFSESK
MIISTPQR
MGMSFGLESGK
TVIDCSISSDK
AQLQELLLQQIAFK
CSLEDLK
WIGLPTNSAQECQNLEIEK
VYDALNVLMAMNIISK
FEYLFNFDNTFEIHDDIEVLK


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.28)
SRM signal intensity rank
ESPPredictor rank

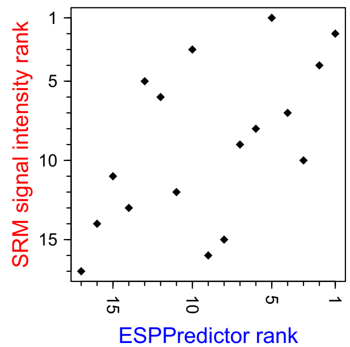

TGIF2
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
ASDVALPR
AEAGSPTGGLFNTPPPTPPEQDK
DPNQFTISR
AAEMELQK
MSDSDLGEDEGLLSLAGK
LLPDMLR
GELESPKPLVTPGSTLTLLTR
YNAYPSEQEK
EDFSSFQLLVEVALQR
DWLYLHR
LSLSGQTNLSVLQICNWFINAR


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.13)
SRM signal intensity rank
ESPPredictor rank

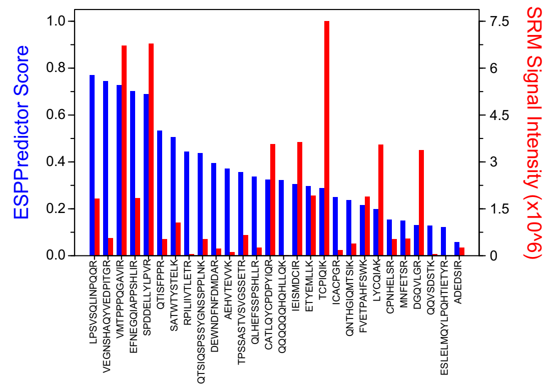
TP53
ESPPredictor vs. Empirical SRM intensities


ESPPredictor Score
SRM Signal Intensity (x10^6)
1.0 0.8 0.6 0.4 0.2 0.0
24.3 19.5 14.6 9.7 4.9 0
CSDSDGLAPPQHLIR
SVTCTYSPALNK
KPLDGEYFTLQIR
ELNEALELK
TCPVQLWVDSTPPPGTR
LGFLHSGTAK
TYQGSYGFR
VEYLDDR
VCACAGR
ALPNNTSSSPQPK
MFCQLAK
QSQHMTEVVR
GEPHHELPSGSTK
MPEAAPR
RPILTIITLEDSSGNLLGR
TEEENLR
AHSSHLK

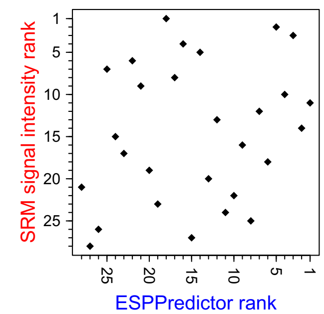
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.56)



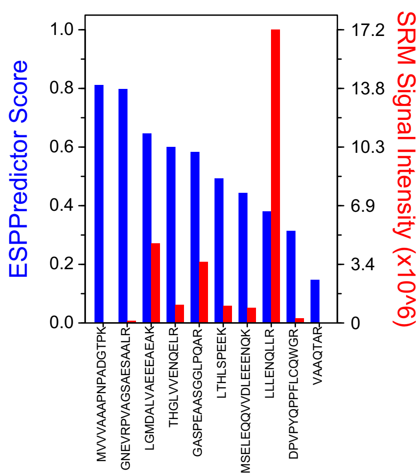
TP73L
ESPPredictor vs. Empirical SRM intensities



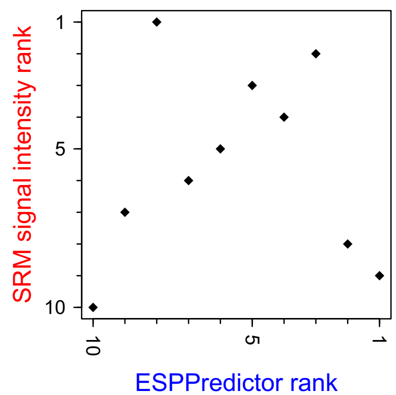
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.23)



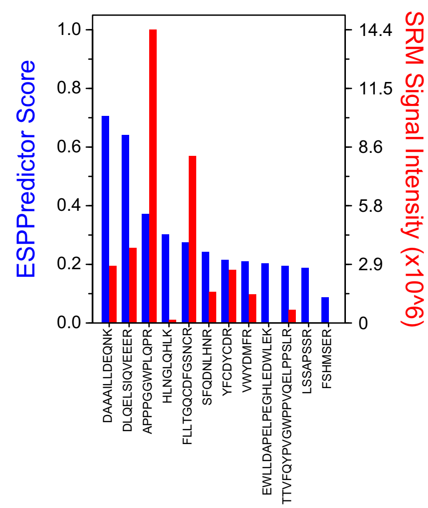
XBP1
ESPPredictor vs. Empirical SRM intensities



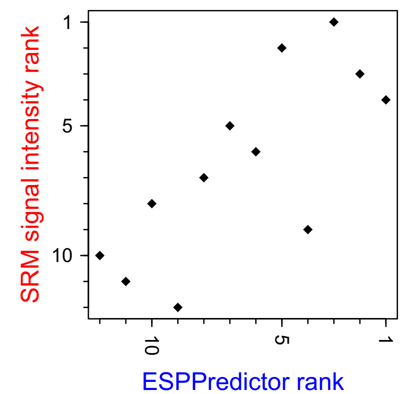
ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.03)

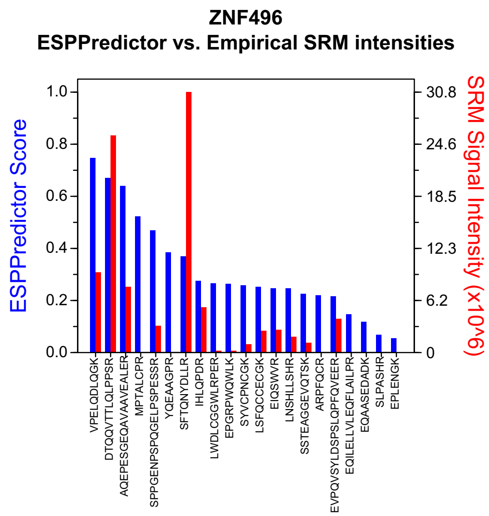


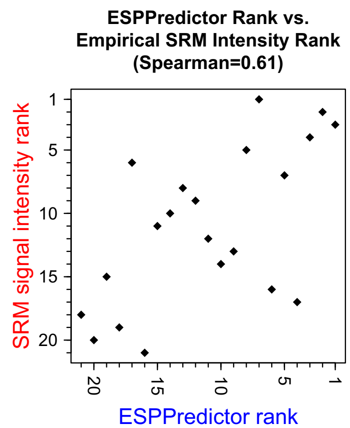
ZMAT5
ESPPredictor vs. Empirical SRM intensities

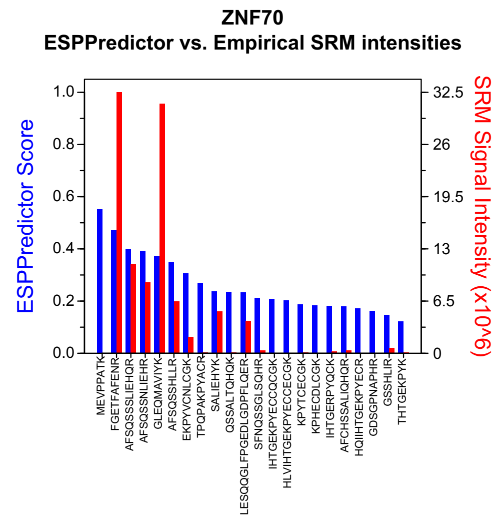

ESPPredictor Score
SRM Signal Intensity (x10^6)
1.0 0.8 0.6 0.4 0.2 0.0
14.4 11.5 8.6 5.8 2.9 0
DAAAILLDEQNK
DLQELSIQVEEER
APPPGGWPLQPR
HLNGLQHLK
FLLTGQCDFGSNCR
SFQDNLHNR
YFCDYCDR
VWYDMFR
EWLLDAPELPEGHLEDWLEK
TTVFQYPVGWPPVQELPPSLR
LSSAPSSR
FSHMSER

ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.76)




ZNF496
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
1.0
0.8
0.6
0.4
0.2
0.0
30.8
24.6
18.5
12.3
6.2
0
VPELQDLQGK
DTQQVTTLQLPPSR
AQEPESGEQAVAVEALER
MPTALCPR
SPPGENPSPQGELPSPESSR
YQEAAGPR
SFTQNYDLLR
IHLQPDR
LWDLCGGWLRPER
EPGRPWQWLK
SYVCPNCGK
LSFQCCECGK
EIQSWVR
LNSHLLSHR
SSTEAGGEVQTSK
ARPFQCR
EVPQVSYLDSPSLQPFQVEER
EQILELLVLEQFLAILPR
EQAASEDADK
SLPASHR
EPLENGK


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.61)
SRM signal intensity rank
ESPPredictor rank
1
5
10
15
20


ZNF70
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
1.0
0.8
0.6
0.4
0.2
0.0
32.5
26
19.5
13
6.5
0
MEVPPATK
FGETFAFENR
AFSQSSSLIEHQR
AFSQSSNLIEHR
GLEQMAVIYK
AFSQSSHLLR
EKPYVCNLCGK
TPQPAKPYACR
SALIEHYK
QSSALTQHQK
LESQQGLFPGEDLGDPFLQER
SFNQSSGLSQHR
IHTGEKPYECQQCGK
HLVIHTGEKPYECECGK
KPYTCECGK
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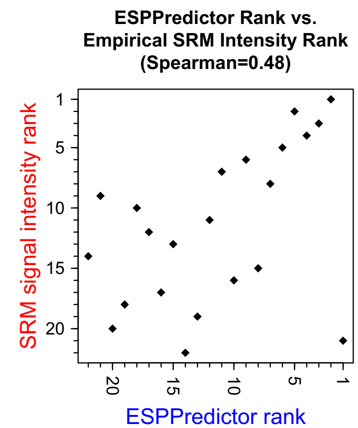
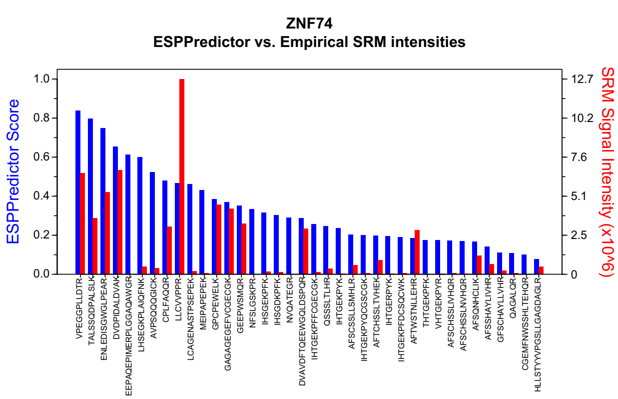
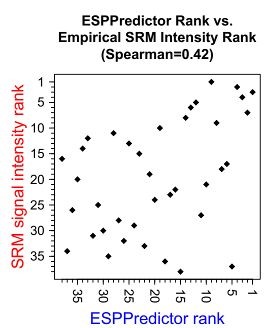
AFCHSSALIQHQR
HQIIHTGEKPYECR
GDSGPNAPHR
GSSHLIR
THTGEKPYK


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.48)
SRM signal intensity rank
ESPPredictor rank
1
5
10
15
20


ZNF74
ESPPredictor vs. Empirical SRM intensities
ESPPredictor Score
SRM Signal Intensity (x10^6)
1.0
0.8
0.6
0.4
0.2
0.0
12.7
10.2
7.6
5.1
2.5
0


ESPPredictor Rank vs.
Empirical SRM Intensity Rank
(Spearman=0.42)
SRM signal intensity rank
ESPPredictor rank
1
5
10
15
20
25
30
35

SUPPLEMENTARY NOTE: TUTORIAL

Skyline Absolute Quantification

Introduction

This tutorial covers how to determine the absolute abundance of a target protein using Selected Reaction Monitoring (SRM) mass spectrometry. Specifically, we will demonstrate how to use an external calibration curve with an internal standard heavy labeled peptide.

Peptide absolute abundance measurements can be obtained using either a single-point or a multiple-point calibration. Single-point calibration absolute abundance measurements are generated by spiking into a target sample a heavy labeled 'standard' version of the target peptide that is of known abundance. The absolute abundance of the 'sample' target peptide is obtained by calculating the relative abundance of the light 'sample' target peptide to the heavy 'standard' target peptide[22]. One drawback is that this approach assumes that a light-to-heavy ratio of 2 implies that the light peptide is actually twice as abundant as the heavy peptide – this is referred to as having a peptide response with a slope of 1. Furthermore, this approach of using a single point calibration makes the assumption that both the light and the heavy peptide are both within the linear range of the mass spectrometry detector. However, these assumptions are not always correct[23,24,25,26].

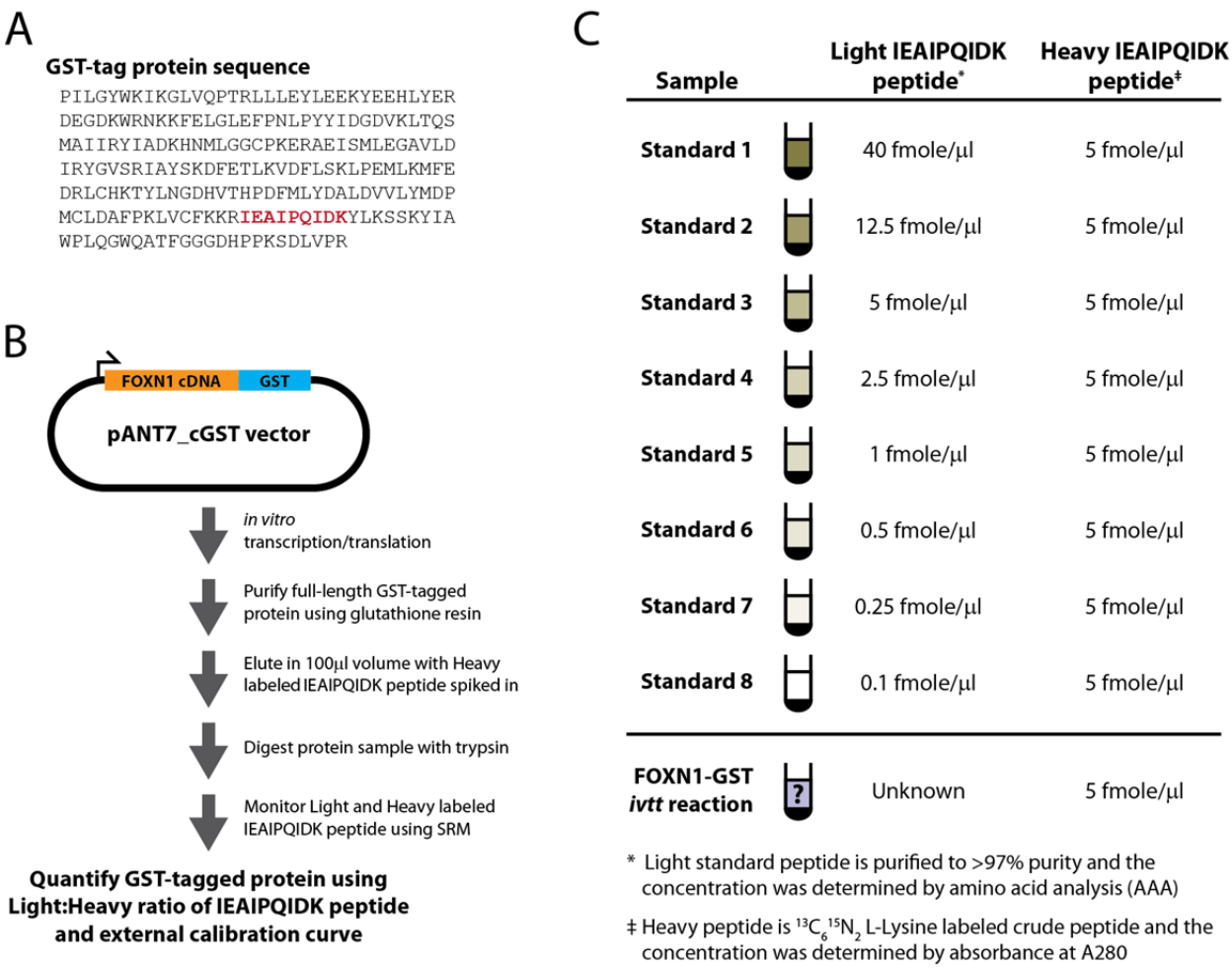
Multiple-point calibration experiments correct for situations where the peptide response does not have a slope of 1. This calibration is done by measuring the signal intensity of a 'standard' peptide at multiple calibration points of known abundance and generating a calibration curve. This calibration curve can then be used to calculate the concentration of the target peptide in a sample, given the signal intensity of that peptide in that sample[24]. One drawback is that this method requires multiple injections into the mass spectrometer to build a calibration curve.

To improve the precision of absolute abundance measurements using an external calibration curve, stable isotope labeled internal standards are often used[27]. Imprecise measurements of the ion intensity of a peptide often arise from sample preparation, autosampler or chromatographic irregularities. By adding an identical quantity of a standard heavy labeled peptide to each of the calibrants and the sample, one is able to measure the ratio of calibrant-to-standard or sample-to-standard. This approach is favored as this ratio is unaffected by some sample preparation, autosampler or chromatographic irregularities. Consequently, by performing peptide absolute quantification using an external calibration curve and an internal standard heavy labeled peptide one is able to obtain the most accurate and precise measurements while minimizing the amount of valuable sample that has to be used.

Experimental Overview

This tutorial will work with data published in Stergachis et al. 2011 where the absolute abundance of GST-tagged proteins were measured using a 'proteotypic' peptide present within the GST-tag (**Tutorial Figure 1A**). For any absolute quantification experiment, it is critical to first identify one or more 'proteotypic' peptides that will be used to quantify the protein of interest. The peptide IEAIPQIDK was identified as 'proteotypic' based on its strong signal intensity relative to other tryptic peptides in the GST-tag (unpublished). Also, this peptide uniquely identifies this schistosomal GST-tag as opposed to other human glutathione-binding proteins.

For this experiment, FOXN1 protein containing an in frame GST-tag was generated using *in vitro* transcription/translation and full-length proteins were purified using glutathione resin (**Tutorial Figure 1B**). Heavy labeled IEAIPQIDK peptide was then spiked into the elution buffer and the sample was digested and analyzed using selected reaction monitoring (SRM) on a Thermo TSQ Vantage triple-quadrupole mass spectrometer. An external calibration curve was generated using different quantities of a light IEAIPQIDK peptide that was purified to >97% purity and the concentration determined by amino acid analysis. Heavy labeled IEAIPQIDK peptide was also spiked into these calibrants at the same concentration as in the FOXN1-GST sample (**Tutorial Figure 1C**). It is important to note that it does not matter what the concentration of the heavy peptide is in each of the samples, so long as it is the same. However, it is best if the amount of heavy peptide in the samples is similar to the amount of light peptide originating from FOXN1-GST. Also, it is best if the concentration of the light peptide originating from FOXN1-GST falls somewhere in the middle of the concentration range tested using the different calibrants.



Tutorial Figure 1. Experimental Overview
(**A**) Schistosomal GST-tag protein sequence. The tryptic peptide used for quantification purposes is indicated in red.
(**B**) Schematic of the synthesis, enrichment, digestion and analysis of tagged proteins.
(**C**) Samples monitored and the abundance of light and heavy IEAIPQIDK peptide in each.

Getting Started

First, please check (https://skyline.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view?name=tutorials) to ensure that this tutorial is up-to-date. Periodic revisions of this tutorial will occur as additional features are added to Skyline.

To start this tutorial, download the following ZIP file:

https://skyline.gs.washington.edu/tutorials/AbsoluteQuant.zip

Extract the files in it to a folder on your computer, like:

C:\Users\absterga\Documents

This will create a new folder:

C:\Users\absterga\Documents\AbsoluteQuant

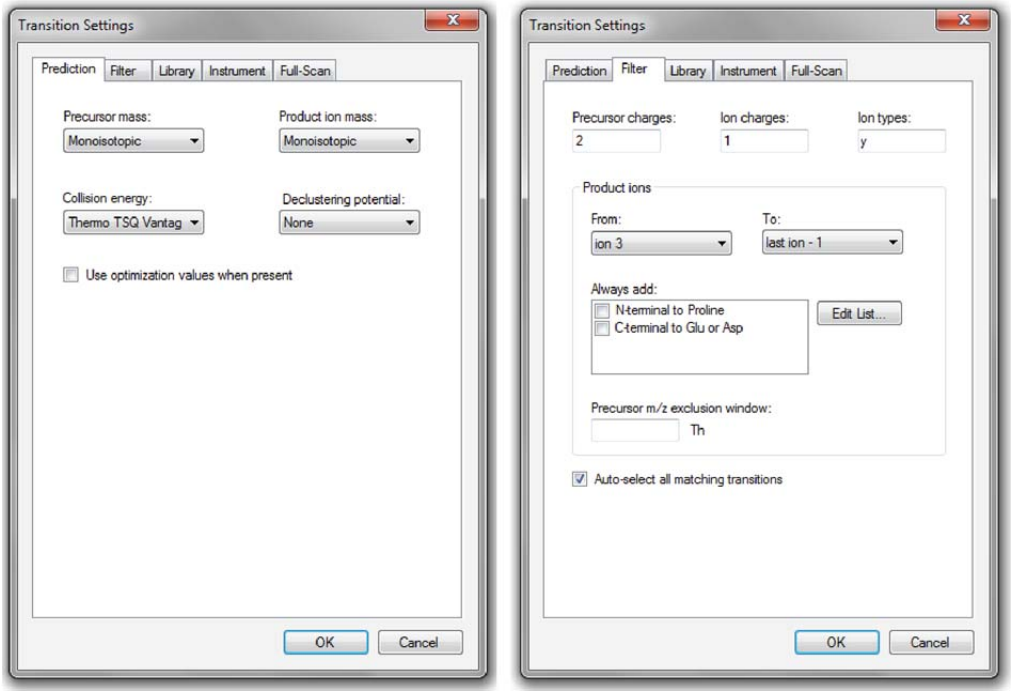
Now start Skyline, and you will be presented with a new empty document.

Generating a Transition List

Before you insert a peptide sequence into Skyline, it is important to make sure that all of the peptide and transition settings are correctly configured for this experiment. The settings described below are designed for $^{13}C_6{}^{15}N_2$ L-Lysine labeled internal standard peptides. If you are using a different isotope, please choose the appropriate isotope modification in the Peptide settings configuration.

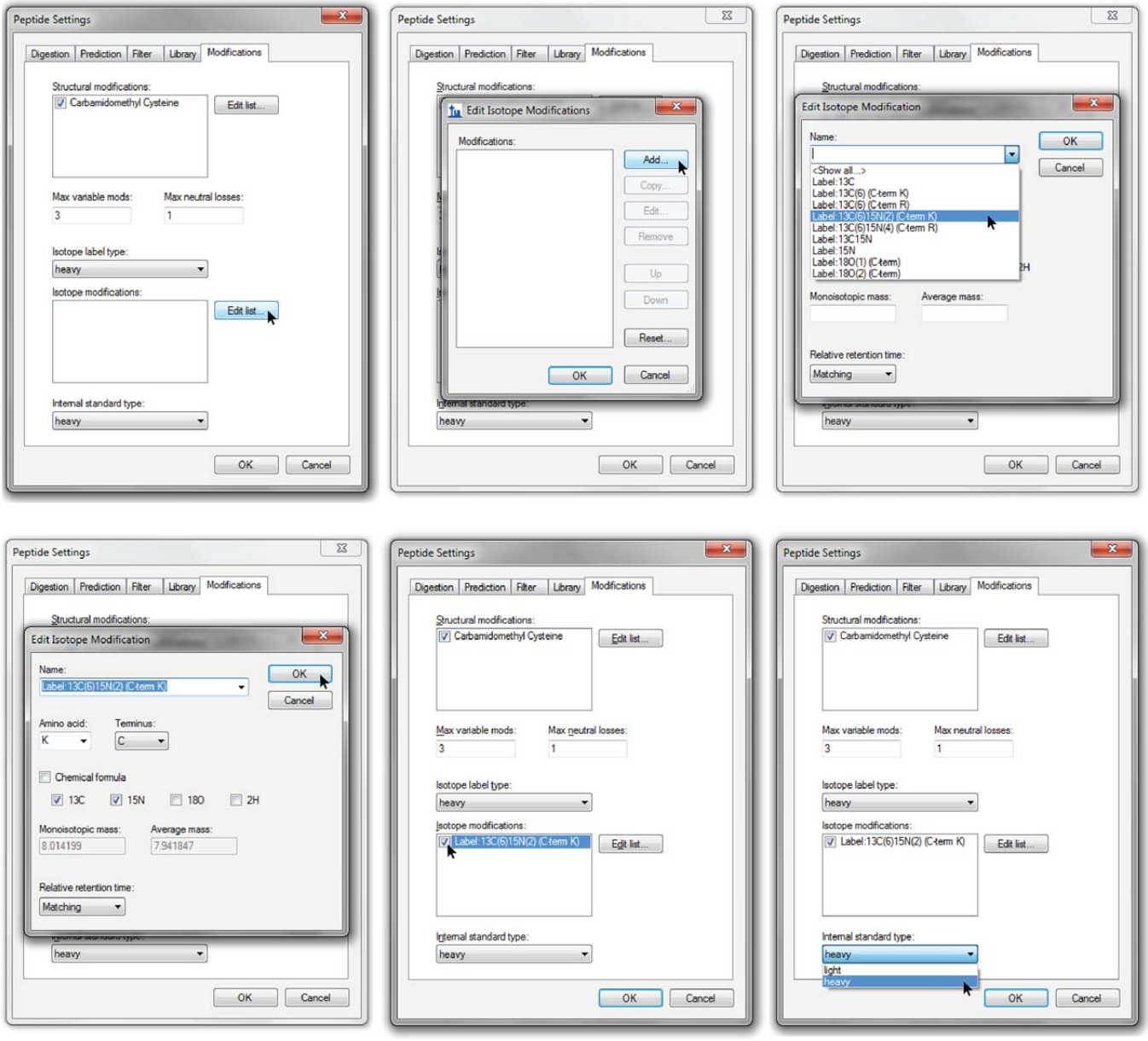
Configuring Transition settings:

- On **Settings** menu, click **Transition Settings**.
- Click the **Prediction** tab.
- Choose **Monoisotopic** for the **Precursor mass** and the **Product ion mass**.
- From the **Collision energy** drop-list choose the instrument that you will be using for your measurements. For this experiment, a **Thermo TSQ Vantage** was used for all measurements.
- Click the **Filter** tab.
- For these experiments we monitored doubly charged precursors (**Precursor charges**), and singly charged (**Ion charges**) y_3 to y_{n-1} product ions (**Ion types** and **Product Ions From** and **To**).
- The correct configuration of these tabs can be seen below.



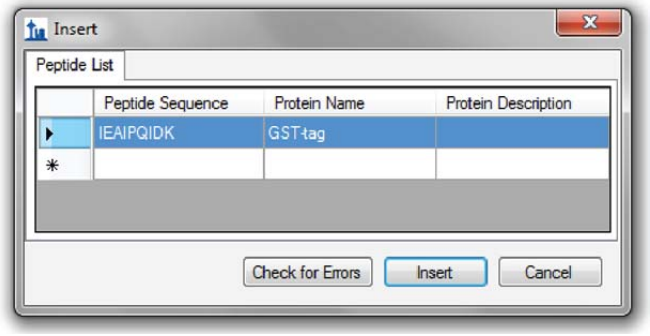
Configuring Peptide settings:

- On the **Settings** menu, click **Peptide Settings**.
- Click the **Modifications** tab.
- Click the **Edit list** button for **Isotope modifications**.
- Click the **Add** button.
- Choose **Label:13C(6)15N(2) (C-term K)** from the **Name** dropdown list.
- Click the **OK** button.
- Check the new **Label:13C(6)15N(2) (C-term K)** modification in the **Isotope modifications** list.
- Since the experiment uses a heavy labeled internal standard peptide, ensure that the **Internal standard type** drop-list is set to **heavy**.

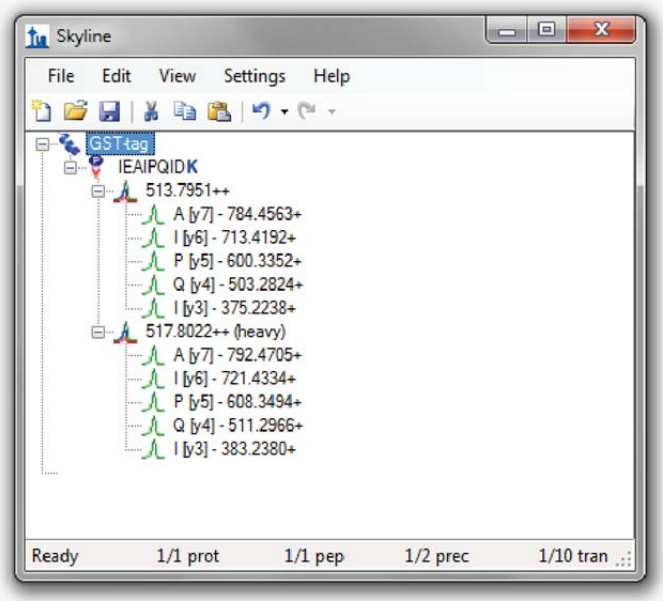


Inserting a peptide sequence:

- On the **Edit** menu, choose **Insert** and click **Peptides**.
- Paste *IEAIPQIDK* into the **Peptide Sequence** box and *GST-tag* into the **Protein Name** box.
- Click the **Insert** button.

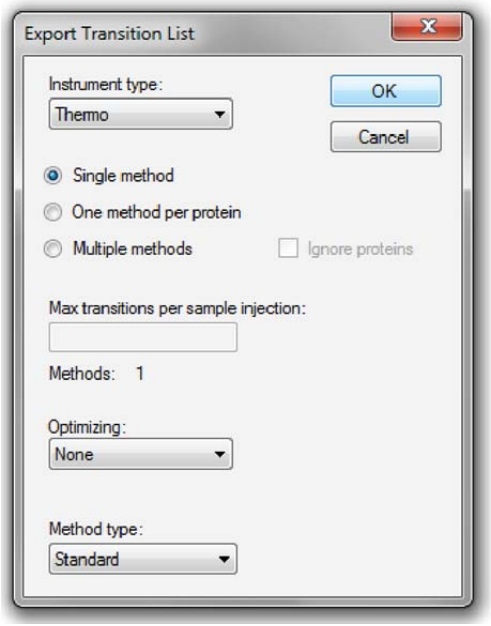


After performing the above steps, the main screen of Skyline should appear as below. You can save this file as test_file or whatever you like in the folder you have created for this tutorial.



Exporting a transition list:

- On the **File** menu, choose **Export** and click **Transition List**.
- The **Export Transition List** form can be configured as desired. Below is what was used for this experiment
- This exported transition list was used to generate an SRM method for a Thermo TSQ Vantage triple-quadrupole mass spectrometer.



Analyzing SRM Data from Calibrants

In this next section you will work with the nine samples indicated in **Tutorial Figure 1C**. You will import the .RAW files into Skyline to view the data. Data will be imported into the saved Skyline document that was generated in the previous section. The files that you will import are contained in the folder you created for this tutorial and are called:

- Standard_1.RAW
- Standard_2.RAW
- Standard_3.RAW
- Standard_4.RAW
- Standard_5.RAW
- Standard_6.RAW
- Standard_7.RAW
- Standard_8.RAW
- FOXN1-GST.RAW

These RAW files were collected in a random order and were interspersed amongst a larger set of runs contained within **Supplemental Data 2** for the original paper (http://proteome.gs.washington.edu/software/skyline/ivt_srm/Supplementary_data_2.zip).

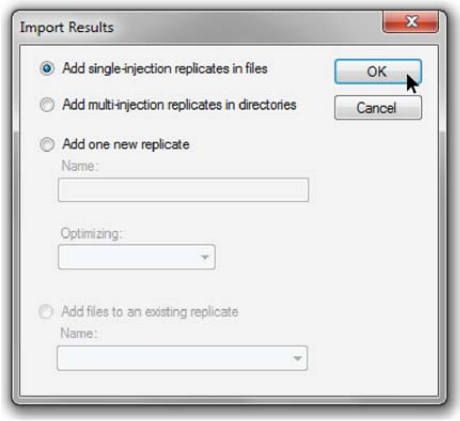
Before you look at the FOXN1-GST sample, you should first become familiar with the standards.

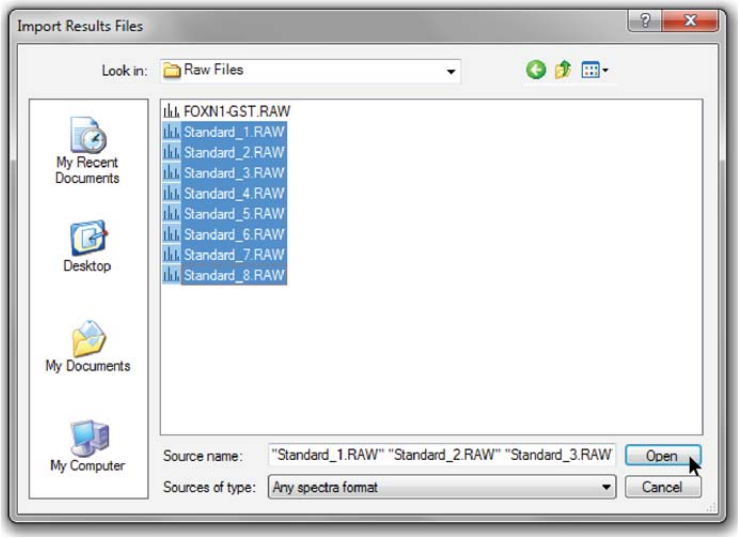
Importing RAW files into Skyline:

- On the **File** menu, choose **Import** and click **Results**.
- Click the **Add single-injection replicates in files** option in the **Import Results** form.

- Click the OK button.
- In the **Import Results Files** form, find and select all eight *Standard* RAW files listed above.
- Click **Open** to import the files.
- When presented with the option to remove the 'Standard_' prefix in creating replicate names, click **Do not remove**.

It may take a few moments for Skyline to import all of the RAW files.

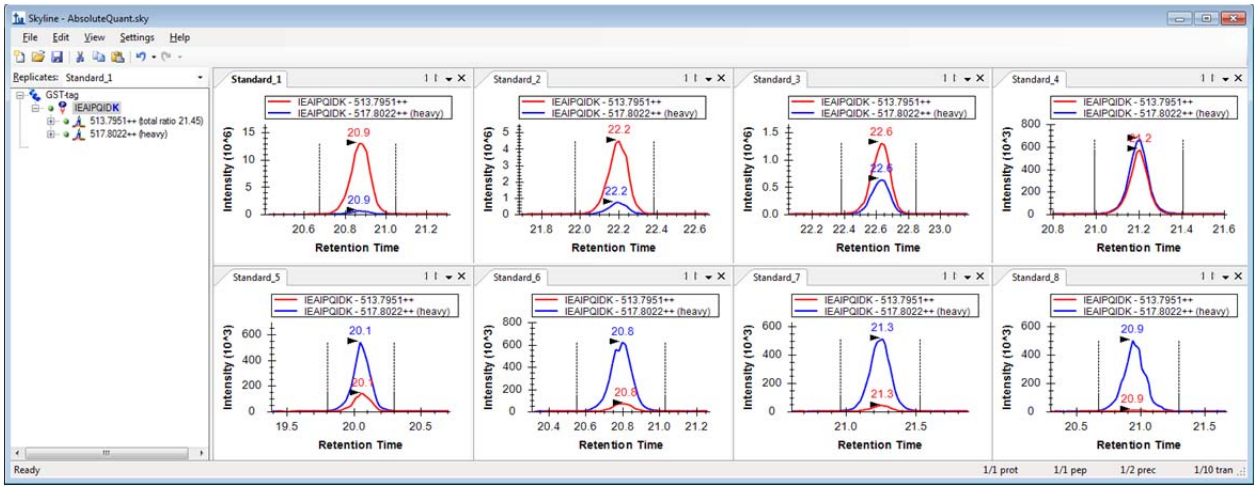




To ensure that the chromatographic peaks for each of the standards looks good, it is best to view all of the traces next to each other in a tiled view.

This can be done by clicking ctrl-T or on the **View** menu, by choosing **Arrange Graphs** and clicking **Tiled**.

If you select the IEAIPQIDK peptide on the left side of the screen, you will see the heavy (Blue) and light (Red) traces loaded into the same window for each standard.



What to inspect when looking at the chromatographic traces for the standards:

- Make sure that the correct peak is selected for both the heavy and light trace of each standard.
- Make sure the peak shapes look Gaussian and do not show an excessively jagged appearance. If this is the case, it may be best to rerun your samples.
- Make sure that the retention time is similar for the different standards. Widely varying retention times often indicate poor chromatography.

Analyzing SRM Data from FOXN1-GST Sample

Next you will want to import the FOXN1-GST.RAW file into the current Skyline document using the same instructions as detailed above. To ensure that this sample looks good, we will inspect the chromatographic trace, the fragmentation pattern and the retention time of both the heavy and light peak.

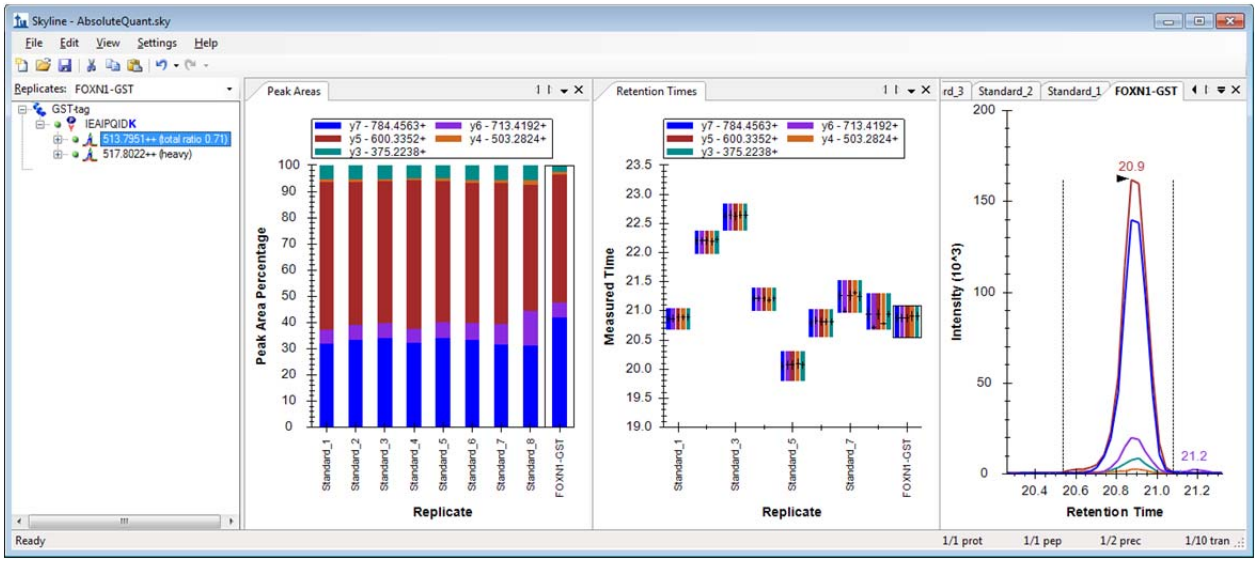
Because this is already a refined method, on the **Settings** menu, click **Integrate All**.

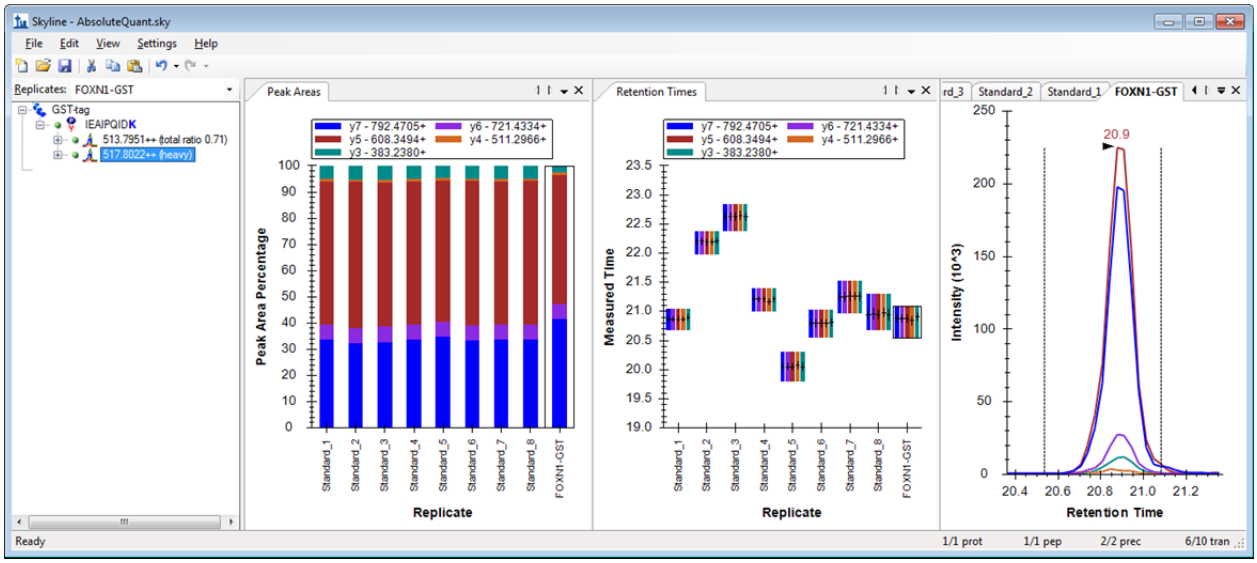
To focus on just the FOXN1-GST data, on the **View** menu, choose **Arrange** and click **Tabbed** (Ctrl-Shift-T), and click on the FOXN1-GST tab.

The **Retention Time** comparison graph can be displayed by pressing F8 or on the **View** menu, by choosing **Retention Times** and then clicking **Replicate Comparison**.

The **Peak Areas** comparison graph can be displayed by pressing F7 or on **View** menu, by choosing **Peak Areas** and then clicking **Replicate Comparison**.

To view the relative contribution of each transition to the total signal intensity, you can right-click on the **Peak Areas** graph, choose **Normalized To** and click **Total.**





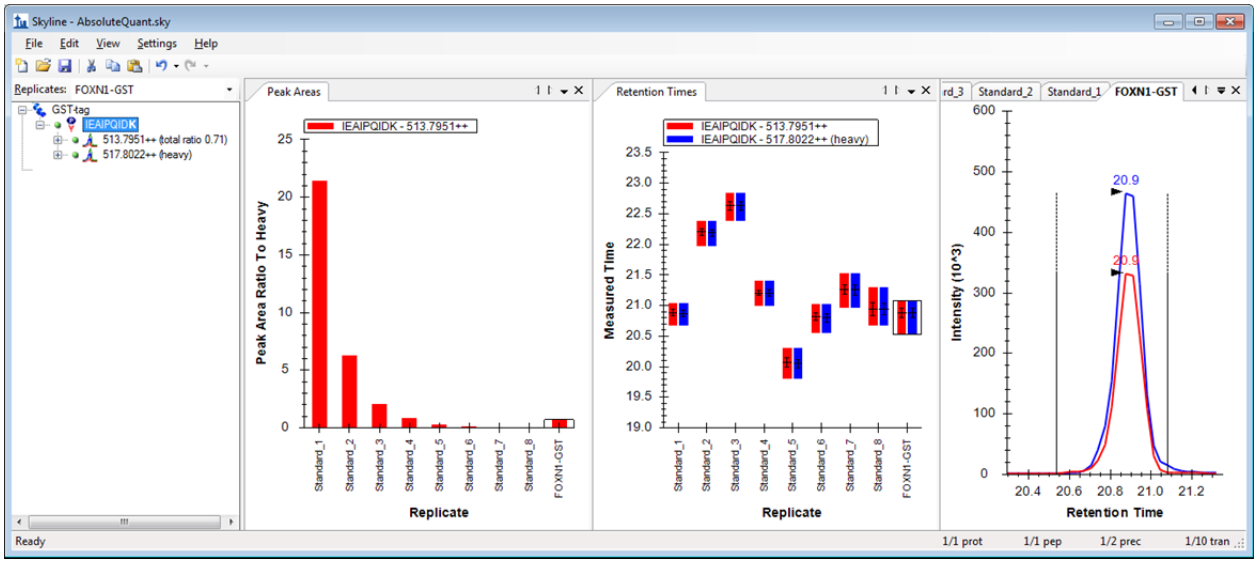
You can then select either the light or heavy precursor and inspect to ensure that:

- The correct peak is selected for both the heavy and light trace.
- The peak shape looks Gaussian and does not show an excessively jagged appearance.
- The retention time is similar for the standards and the FOXN1-GST sample.
- The relative contribution of each transition to the total signal is similar for each sample. If this does not appear to be so, then an incorrect peak is likely selected for one of the samples.

Another way to view the data is to select the IEAIPQIDK peptide in the Peptide View to the left, as opposed to the individual light and heavy precursor ions.

To view the light-to-heavy ratio for each standard and the FOXN1-GST sample, you can right-click on the **Peak Areas** graph, choose **Normalized To** and click **Heavy.**

The values displayed in this **Peak Areas** graph will be the ones we use to build our calibration curve. It can be easily observed from this graph that the light-to-heavy ratio for the FOXN1-GST sample falls somewhere in the middle of the ratios from our calibration points. This is ideal, as this portion of the calibration curve is best for quantification purposes.

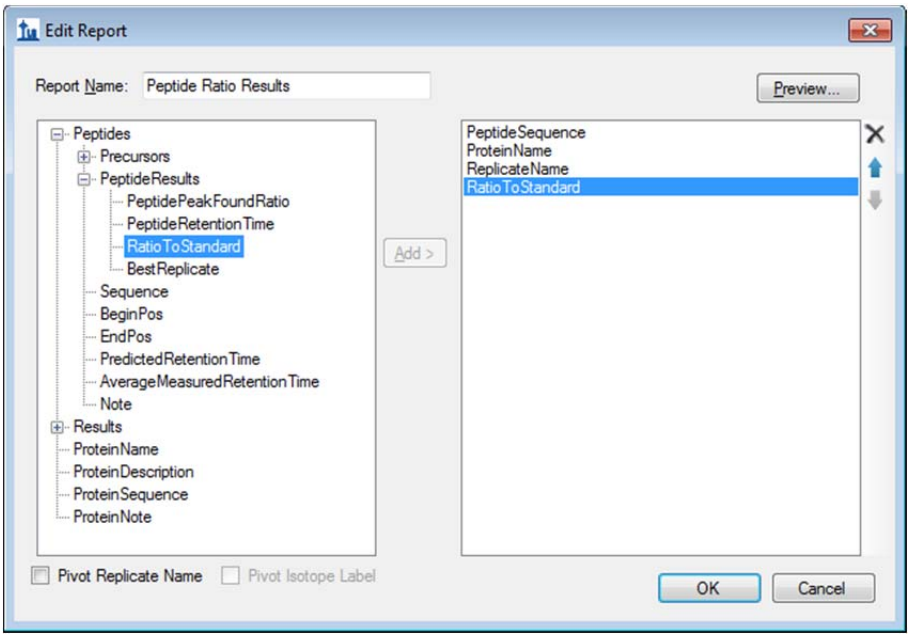


Generating a Calibration Curve

For this tutorial, you will make a calibration curve using Microsoft Excel. However, this can be done using other graphical or statistical programs such as R.

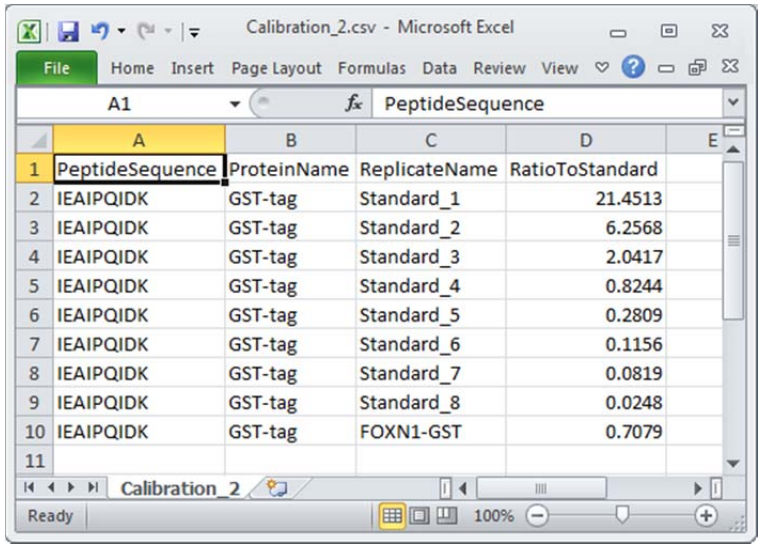
Exporting data from Skyline:

- On the **File** menu, choose **Export** and click **Report**.
- Click **Edit List** and then **Add** to specify the columns that will appear in the report.
- For this experiment you will want to have identifying features for each sample, as well as the **RatioToStandard** (See below for the parameters included).
- Once the report is configured, you can provide a **Report Name**.



- Click **OK**.
- Click **OK** in the **Edit Reports** form.
- Click **Export** in the **Export Report** form.
- Enter 'Calibration.csv' in the **File name** field of the save form that appears.
- Click **Save**.

When opened in Excel, the exported report should look like this:



	A	B	C	D
1	PeptideSequence	ProteinName	ReplicateName	RatioToStandard
2	IEAIPQIDK	GST-tag	Standard_1	21.4513
3	IEAIPQIDK	GST-tag	Standard_2	6.2568
4	IEAIPQIDK	GST-tag	Standard_3	2.0417
5	IEAIPQIDK	GST-tag	Standard_4	0.8244
6	IEAIPQIDK	GST-tag	Standard_5	0.2809
7	IEAIPQIDK	GST-tag	Standard_6	0.1156
8	IEAIPQIDK	GST-tag	Standard_7	0.0819
9	IEAIPQIDK	GST-tag	Standard_8	0.0248
10	IEAIPQIDK	GST-tag	FOXN1-GST	0.7079
11				

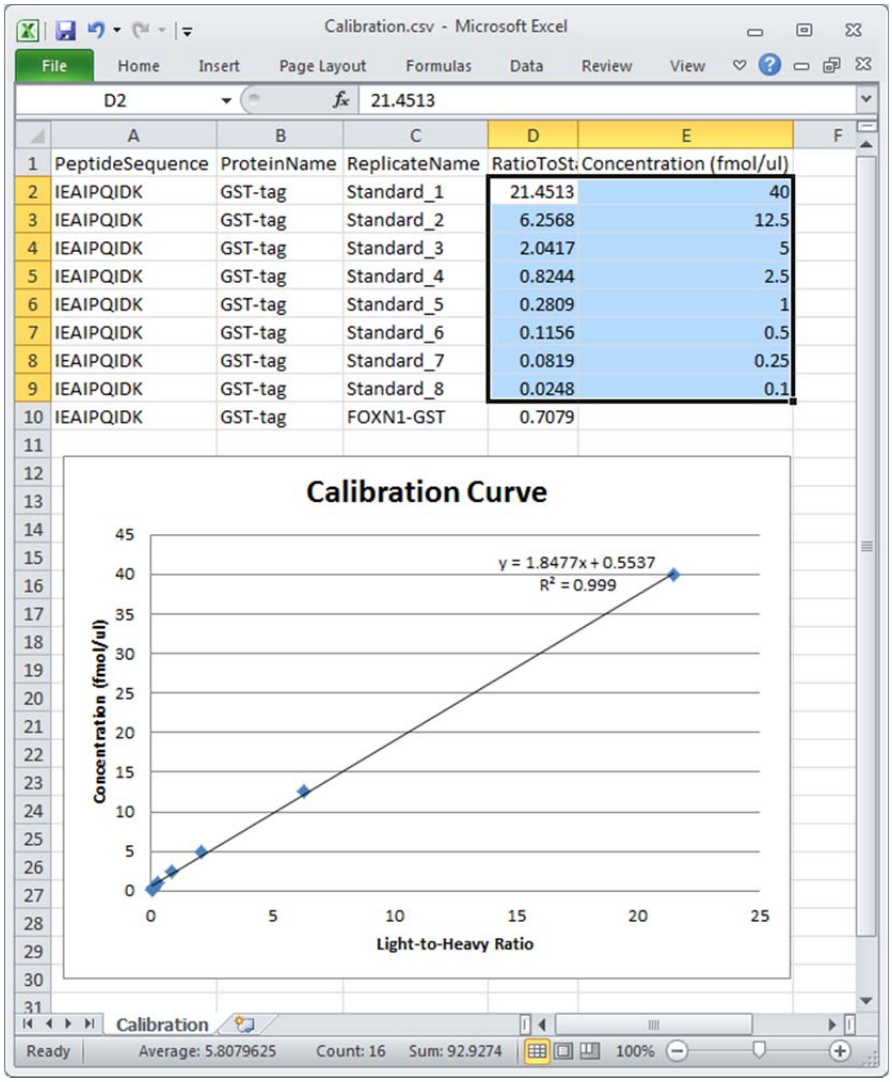
To generate a calibration curve, you first need to specify for each standard the concentration of the light IEAIPQIDK peptide. These values can be found in **Tutorial Figure 1C** and are also displayed below.

Once these known concentrations are entered, you can display the calibration curve by selecting the values, as indicated below, and clicking the **Insert** tab and then **Scatter Plot**.

A trendline for the calibration curve can be generated by right-clicking on the data points within the graph and selecting **Add Trendline**. From the trendline options, you will want to select Linear Regression. In more recent versions of Excel, you will also want to make sure the check boxes are checked to display the equation and the R-squared value on the chart.

Now inspect the trendline to ensure that:

- None of the standards drastically depart from it.
- The data points still appear to follow the trendline even at the lower concentration points.
- The R^2 value is high, indicating a good fit.



Calculating the Concentration of the FOXN1-GST Sample

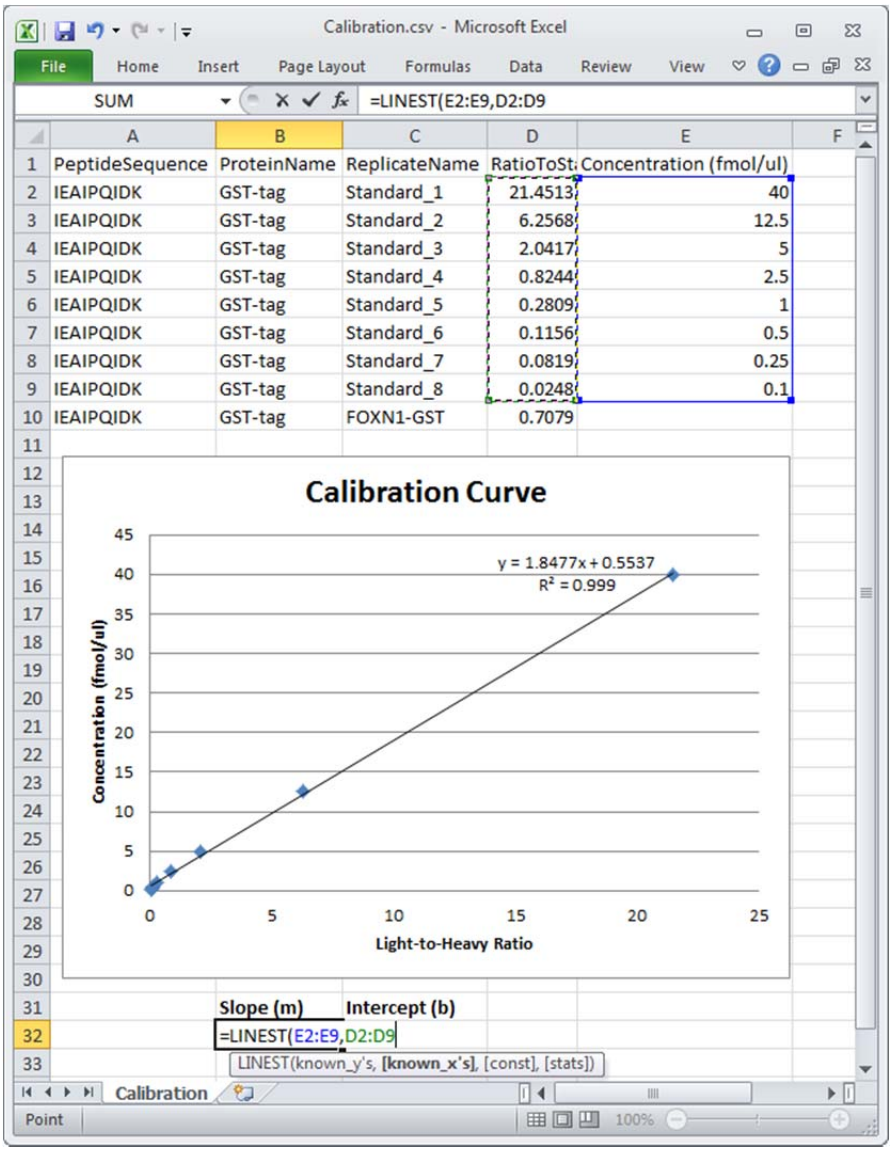
To calculate the concentration of the light IEAIPQIDK peptide within the FOXN1-GST sample you will use the calibration curve from the previous section. This will allow you to calibrate the light-to-heavy ratio of IEAIPQIDK within the FOXN1-GST sample to known concentrations. To do this, you must first identify the slope and intercept of the calibration curve.

To do this, you can select two adjacent cells in Excel and type into the equation dialog:

=LINEST(E2:E9,D2:D9)

E2:E9 are the y-values (Concentration)
D2:D9 are the x-values (light-to-heavy ratio)

Then press **ctrl-shift-Enter** and the Slope and Intercept will be displayed in the two selected cells

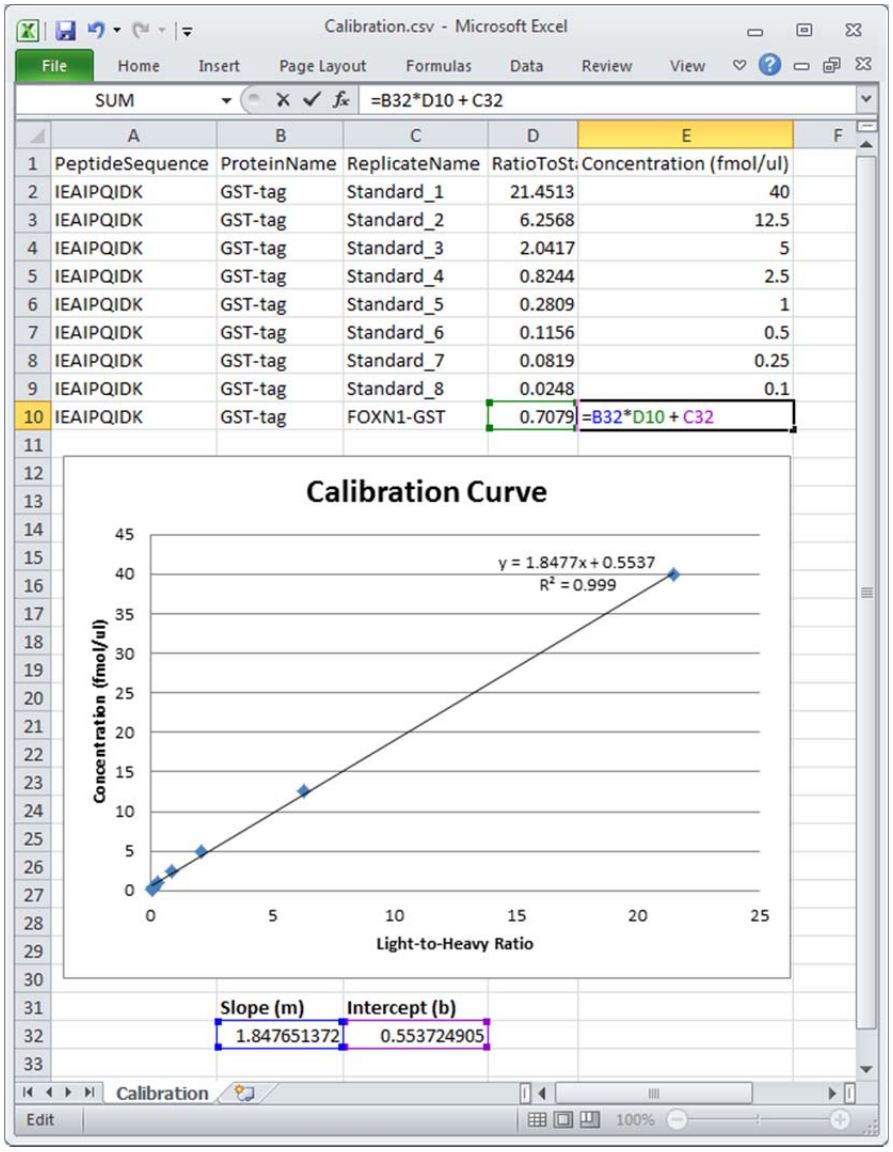


Using this linear equation the concentration of any unknown sample (y-value) can be obtained by inserting the light-to-heavy ratio of that sample (x-value) into a standard **y = m*x + b** equation (*concentration = slope * ratio + intercept*).

We can do this for the FOXN1-GST sample in excel by typing into a cell:

=B32*D10 + C32

B32 is the slope of the linear regression
D10 is the light-to-heavy ratio of the FOXN1-GST sample
C32 is the Intercept of the linear regression



This yields the value 1.86 as the concentration of FOXN1-GST within our sample. This concentration is in units fmole/µl or nM, as this is the concentration of our calibrants used for the linear regression.

Since the purified FOXN1-GST reaction was resuspended in 100 µl total volume, we can state that there are 189 femtomoles, or $1.13*10^{11}$ molecules of FOXN1-GST within our sample.

Conclusion

This tutorial presented the advantages of different absolute abundance experimental setups and demonstrated how to determine absolute abundances using an external calibration curve with an internal standard heavy labeled peptide. This method provides accurate and precise absolute measurements while minimizing the amount of valuable sample that has to be used during the experiment.

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