

Supplementary Materials: A Comprehensive Analysis of FUT8 Overexpressing Prostate Cancer Cells Reveals the Role of EGFR in Castration Resistance

Supplemental Figure 1

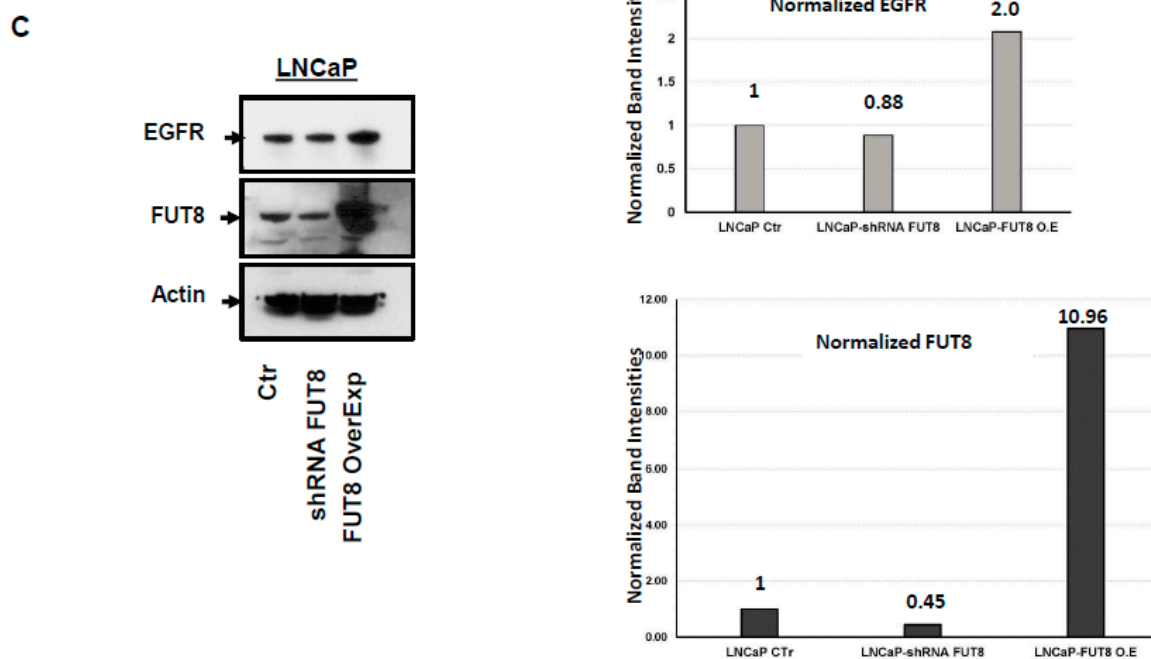


Figure S1. Western blot analysis for phosphorylated EGFR protein in LNCaP Ctr and LNCaP-FUT8 cells that were either mock treated (DMSO) or treated with EGF (2ng/ml). Actin is included to serve as equal amount of loading across the lanes (A). Differential expression of several phospho-peptides identified across cell lines when compare to the wildtype LNCaP-Ctr cells (B). Western blot analysis for the LNCaP Ctr and LNCaP-shRNA Fut8 cells showing downregulation of EGFR (C).

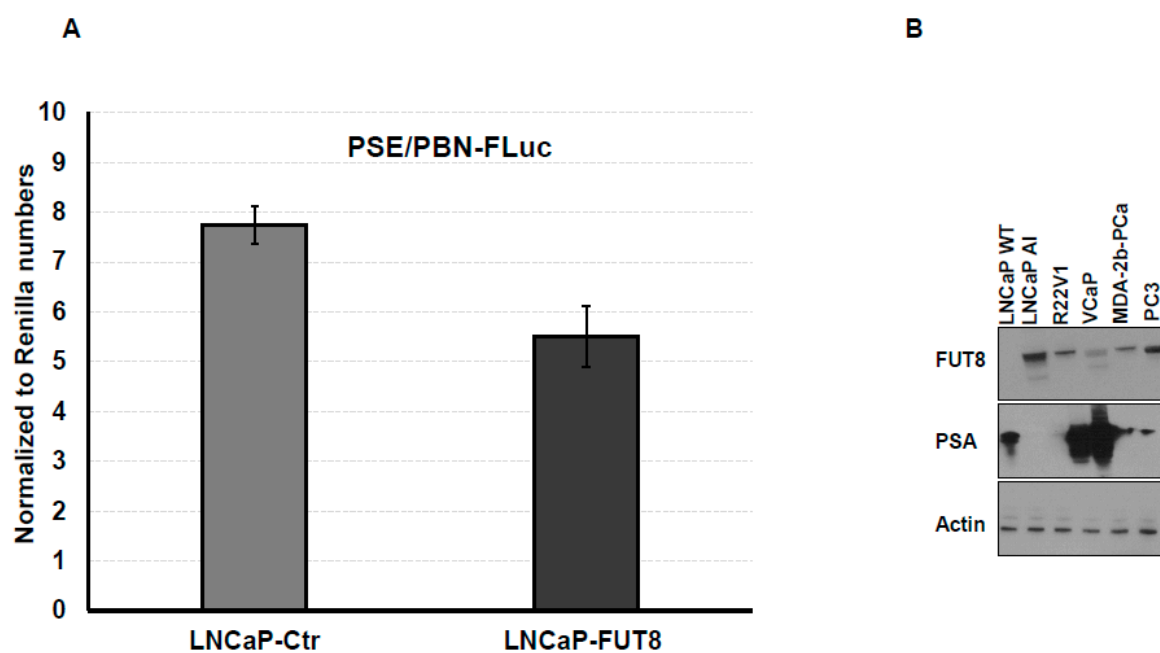


Figure S2. AR reporter assay showing the activity of PSE/PBN promoter in LNCaP Ctr and LNCaP-FUT8 cells (A) Western blot analysis for FUT8 and PSA in several different prostate cancer cell lines (B).

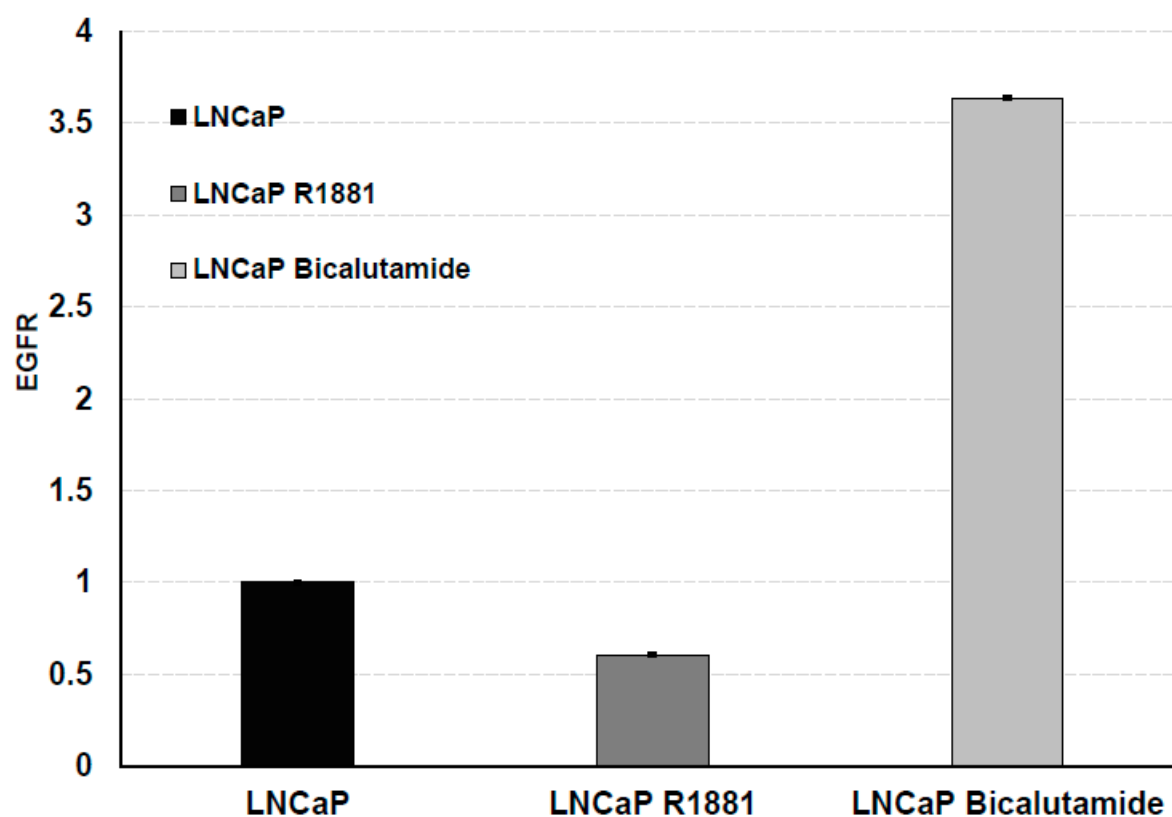


Figure S3. QRT PCR analysis showing relative expression of EGFR mRNA in cells that were treated with R1881 or with anti-androgen (Bicalutamide).

A Intensity ratio of each band Normalized to Actin Intensity

	LNCaP WT	LNCaP-FUT8
FUT8	0.446	0.863
EGFR	0.517	0.665
AR	0.757	0.801
PSA	1.116	0.847

Figure S4. Quantitation of western blot using ImageJ in LNCaP WT and LNCaP-FUT8 for figure 3A and B.

Supplemental Table 3a

Index	Label	log2(LNCaP-FUT8)	log2(PC3)	log2(LNCaP-95)	RowLabel	High_Man	Static	Fuc	Others	GlyLabel
DSISINATNIK_N2H10	N352_HexNAc(2)Hex(10)	2.281536508	1.745930216	1.544194624	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H4	N352_HexNAc(2)Hex(4)	0.157335282	0.863108056	1.191806893	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H5	N352_HexNAc(2)Hex(5)	0.396228836	0.397908839	0.534980489	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H5F1	N352_HexNAc(2)Hex(5)Fuc(1)	0.347006872	0.106117502	0.190493903	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H6	N352_HexNAc(2)Hex(6)	0.661104339	0.634133998	0.256901508	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H6F1	N352_HexNAc(2)Hex(6)Fuc(1)	2.033087438	2.271206919	0.266873208	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H7	N352_HexNAc(2)Hex(7)	2.377751257	2.845200045	0.587646452	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H7F1	N352_HexNAc(2)Hex(7)Fuc(1)	1.954272233	2.097150725	0.257012574	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H8	N352_HexNAc(2)Hex(8)	1.968219964	1.882512616	0.043141685	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H9	N352_HexNAc(2)Hex(9)	1.77972981	1.611446823	0.981005875	EGFR	1	0	0	0	High_Man
DSISINATNIK_N4H4S1	N352_HexNAc(4)Hex(4)NeuAc(1)	1.174165198	1.277168108	-0.286061636	EGFR	0	1	0	0	Static
DSISINATNIK_N4H4F1	N352_HexNAc(4)Hex(4)Fuc(1)	0.520727072	0.572157631	0.54298122	EGFR	0	0	1	0	Fucose
DSISINATNIK_N4H4F2	N352_HexNAc(4)Hex(4)Fuc(2)	1.483874327	1.72051809	0.174441385	EGFR	0	0	1	0	Fucose
DSISINATNIK_N6H4	N352_HexNAc(6)Hex(4)	1.591847009	1.636491822	0.909359565	EGFR	0	0	0	1	Others
DSISINATNIK_N6H5	N352_HexNAc(6)Hex(5)	1.562503669	1.021037045	0.953288895	EGFR	0	0	0	1	Others
NCTSGDLHILPVAFR_N2H6	N361_HexNAc(2)Hex(6)	-0.089733346	-0.881571903	-0.546584611	EGFR	1	0	0	0	High_Man
NCTSGDLHILPVAFR_N2H7	N361_HexNAc(2)Hex(7)	-0.303394753	-0.524193275	-0.857775485	EGFR	1	0	0	0	High_Man
NCTSGDLHILPVAFR_N2H8	N361_HexNAc(2)Hex(8)	0.029247539	-0.310308457	-0.148136411	EGFR	1	0	0	0	High_Man
NCTSGDLHILPVAFR_N4H4F1	N361_HexNAc(4)Hex(4)Fuc(1)	0.268611573	-0.018577045	0.180085311	EGFR	0	0	1	0	Fucose
NCTSGDLHILPVAFR_N4H5F1	N361_HexNAc(4)Hex(5)Fuc(1)	0.494577845	0.465247283	0.097712162	EGFR	0	0	1	0	Fucose
NCTSGDLHILPVAFR_N4H6F1	N361_HexNAc(4)Hex(6)Fuc(1)	1.290284143	1.311640091	0.445364372	EGFR	0	0	1	0	Fucose
NCTSGDLHILPVAFR_N4H7F1	N361_HexNAc(4)Hex(7)Fuc(1)	0.636479126	0.94183678	0.519462108	EGFR	0	0	1	0	Fucose
LLIQAWPENR_N4H5F1	N413_HexNAc(4)Hex(5)Fuc(1)	-0.02366056	-1.031971808	-0.605217375	EGFR	0	0	1	0	Fucose
LLIQAWPENR_N4H5F2	N413_HexNAc(4)Hex(5)Fuc(2)	0.24825543	-0.742616566	-0.085881182	EGFR	0	0	1	0	Fucose
TCPAGVMGNNNTLVWK_N2H12	N603_HexNAc(2)Hex(12)	-0.126125358	0.163035408	1.21288094	EGFR	1	0	0	0	High_Man
TCPAGVMGNNNTLVWK_N2H8	N603_HexNAc(2)Hex(8)	0.81621641	0.90815393	0.2203077	EGFR	1	0	0	0	High_Man
TCPAGVMGNNNTLVWK_N4H5F1	N603_HexNAc(4)Hex(5)Fuc(1)	0.671891766	0.032315783	0.527900002	EGFR	0	0	1	0	Fucose
TCPAGVMGNNNTLVWK_N4H5F1S1	N603_HexNAc(4)Hex(5)Fuc(1)NeuAs(1)	0.670303892	1.275141235	2.606762278	EGFR	0	1	1	0	Static
TCPAGVMGNNNTLVWK_N4H5F3	N603_HexNAc(4)Hex(5)Fuc(3)	0.340046573	0.439915604	1.674639367	EGFR	0	0	1	0	Fucose
TCPAGVMGNNNTLVWK_N6H7	N603_HexNAc(6)Hex(7)	-0.043013751	0.355739112	1.569981046	EGFR	0	0	0	1	Others
YADAGHVCHLCHPNCTYGTGPGLEGCTNGPK_N2H5	N623_HexNAc(2)Hex(5)	0.509694414	0.22595487	0.283675536	EGFR	1	0	0	0	High_Man
YADAGHVCHLCHPNCTYGTGPGLEGCTNGPK_N2H6	N623_HexNAc(2)Hex(6)	1.00998893	0.430777198	-0.302030653	EGFR	1	0	0	0	High_Man
YADAGHVCHLCHPNCTYGTGPGLEGCTNGPK_N3H5F1	N623_HexNAc(3)Hex(5)Fuc(1)	1.397379953	1.292584461	0.18147891	EGFR	0	0	1	0	Fucose
YADAGHVCHLCHPNCTYGTGPGLEGCTNGPK_N4H4F1	N623_HexNAc(4)Hex(4)Fuc(1)	0.708429231	0.297910351	-0.425135388	EGFR	0	0	1	0	Fucose

Supplemental Table 3b

Index	Label	log ₂ (LNCA-P-Fu8)	log ₂ (PC3)	log ₂ (LNCA-P-95)	RowLabel	High_Man	Sialic	Fuc	Others	GlyLabel
DSISINATNIK_N2H10	N352_HexNAc(2)Hex(10)	2.291633	1.74699	1.544199	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H4	N352_HexNAc(2)Hex(4)	0.157339	0.983109	1.191807	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H6	N352_HexNAc(2)Hex(6)	0.986229	0.387909	0.534889	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H5F1	N352_HexNAc(2)Hex(5)Fuc(1)	0.347007	0.106118	0.190494	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H8	N352_HexNAc(2)Hex(8)	0.981104	0.834134	0.258932	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H6F1	N352_HexNAc(2)Hex(6)Fuc(1)	2.033087	2.271207	0.266873	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H7	N352_HexNAc(2)Hex(7)	2.97773	2.8463	0.687366	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H7F1	N352_HexNAc(2)Hex(7)Fuc(1)	1.954272	2.097151	0.257013	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H8	N352_HexNAc(2)Hex(8)	1.86822	1.882519	0.545142	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H9	N352_HexNAc(2)Hex(9)	1.77973	1.811443	0.891006	EGFR	1	0	0	0	High_Man
DSISINATNIK_N2H4F3	N352_HexNAc(4)Hex(4)Fuc(3)	0.598807	0.611492	0.204886	EGFR	0	0	1	0	Fucose
DSISINATNIK_N4H4S1	N352_HexNAc(4)Hex(4)NeuAc(1)	1.174186	1.277188	-0.288086	EGFR	0	1	0	0	Sialic
DSISINATNIK_N4H4F1	N352_HexNAc(4)Hex(4)Fuc(1)	0.520727	0.572158	0.542981	EGFR	0	0	1	0	Fucose
DSISINATNIK_N4H4F2	N352_HexNAc(4)Hex(4)Fuc(2)	1.493874	1.720518	0.174441	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N2H8	N361_HexNAc(2)Hex(8)	-0.08973	-0.88187	-0.546582	EGFR	1	0	0	0	High_Man
NCT518GDLHLPVAFR_N2H7	N361_HexNAc(2)Hex(7)	0.303386	-0.62418	-0.867789	EGFR	1	0	0	0	High_Man
NCT518GDLHLPVAFR_N2H6	N361_HexNAc(2)Hex(6)	0.026048	-0.3103	-0.148146	EGFR	1	0	0	0	High_Man
NCT518GDLHLPVAFR_N4H4F1	N361_HexNAc(4)Hex(4)Fuc(1)	0.288912	-0.01868	0.180085	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N4H5F1	N361_HexNAc(4)Hex(5)Fuc(1)	0.484578	0.466247	0.097712	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N4H6F1	N361_HexNAc(4)Hex(6)Fuc(1)	1.290264	1.31164	0.449364	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N4H7F1	N361_HexNAc(4)Hex(7)Fuc(1)	0.636479	0.941837	0.519462	EGFR	0	0	1	0	Fucose
LLIQAWPENR_N4H5F1	N413_HexNAc(4)Hex(5)Fuc(1)	-0.02366	-1.03197	-0.60522	EGFR	0	0	1	0	Fucose
LLIQAWPENR_N4H5F2	N413_HexNAc(4)Hex(5)Fuc(2)	0.248255	-0.74262	-0.08588	EGFR	0	0	1	0	Fucose
TCPAGVMGENTLVWK_N2H12	N603_HexNAc(2)Hex(12)	-0.12613	0.183036	1.212881	EGFR	1	0	0	0	High_Man
TCPAGVMGENTLVWK_N2H8	N603_HexNAc(2)Hex(8)	0.818216	0.908164	0.220308	EGFR	1	0	0	0	High_Man
TCPAGVMGENTLVWK_N4H5F1	N603_HexNAc(4)Hex(5)Fuc(1)	0.671892	0.032316	0.5279	EGFR	0	0	1	0	Fucose
TCPAGVMGENTLVWK_N4H5F1S1	N603_HexNAc(4)Hex(5)Fuc(1)NeuAc(1)	0.670304	1.275141	2.606762	EGFR	0	1	1	0	Sialic
TCPAGVMGENTLVWK_N4H5F3	N603_HexNAc(4)Hex(5)Fuc(3)	0.340047	0.439916	1.674638	EGFR	0	0	1	0	Fucose
YADAGHVCHLCPNCTYOGTGP0LEOG PTNGPK_N2H6	N623_HexNAc(3)Hex(6)Fuc(1)	0.609884	0.226866	0.283878	EGFR	1	0	0	0	High_Man
YADAGHVCHLCPNCTYOGTGP0LEOG PTNGPK_N2H8	N623_HexNAc(3)Hex(8)Fuc(1)	1.009988	0.430777	-0.302038	EGFR	1	0	0	0	High_Man
YADAGHVCHLCPNCTYOGTGP0LEOG PTNGPK_N4H5F1	N623_HexNAc(4)Hex(5)Fuc(1)	1.39738	1.292584	0.181479	EGFR	0	0	1	0	Fucose
YADAGHVCHLCPNCTYOGTGP0LEOG PTNGPK_N4H4F1	N623_HexNAc(4)Hex(4)Fuc(1)	0.708429	0.29791	-0.42514	EGFR	0	0	1	0	Fucose

Supplemental Table 3c

Index	Label	log ₂ (LNCA-P-Fu8)	log ₂ (PC3)	log ₂ (LNCA-P-95)	RowLabel	High_Man	Sialic	Fuc	Others	GlyLabel
DSISINATNIK_N2H5F1	N352_HexNAc(2)Hex(5)Fuc(1)	0.347007	0.106118	0.190494	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H6F1	N352_HexNAc(2)Hex(6)Fuc(1)	2.033087	2.271207	0.266873	EGFR	0	0	1	0	Fucose
DSISINATNIK_N2H7F1	N352_HexNAc(2)Hex(7)Fuc(1)	1.954272	2.097151	0.257013	EGFR	0	0	1	0	Fucose
DSISINATNIK_N4H4F1	N352_HexNAc(4)Hex(4)Fuc(1)	0.520727	0.572158	0.542981	EGFR	0	0	1	0	Fucose
DSISINATNIK_N4H4F2	N352_HexNAc(4)Hex(4)Fuc(2)	1.493874	1.720518	0.174441	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N4H4F1	N361_HexNAc(4)Hex(4)Fuc(1)	0.288912	-0.01868	0.180085	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N4H5F1	N361_HexNAc(4)Hex(5)Fuc(1)	0.484578	0.466247	0.097712	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N4H6F1	N361_HexNAc(4)Hex(6)Fuc(1)	1.290264	1.31164	0.449364	EGFR	0	0	1	0	Fucose
NCT518GDLHLPVAFR_N4H7F1	N361_HexNAc(4)Hex(7)Fuc(1)	0.636479	0.941837	0.519462	EGFR	0	0	1	0	Fucose
LLIQAWPENR_N4H5F1	N413_HexNAc(4)Hex(5)Fuc(1)	-0.02366	-1.03197	-0.60522	EGFR	0	0	1	0	Fucose
LLIQAWPENR_N4H5F2	N413_HexNAc(4)Hex(5)Fuc(2)	0.248255	-0.74262	-0.08588	EGFR	0	0	1	0	Fucose
TCPAGVMGENTLVWK_N4H5F1	N603_HexNAc(4)Hex(5)Fuc(1)	0.671892	0.032316	0.5279	EGFR	0	0	1	0	Fucose
TCPAGVMGENTLVWK_N4H5F1S1	N603_HexNAc(4)Hex(5)Fuc(1)NeuAc(1)	0.670304	1.275141	2.606762	EGFR	0	1	1	0	Sialic
TCPAGVMGENTLVWK_N4H5F3	N603_HexNAc(4)Hex(5)Fuc(3)	0.340047	0.439916	1.674638	EGFR	0	0	1	0	Fucose
YADAGHVCHLCPNCTYOGTGP0LEOG PTNGPK_N3H5F1	N623_HexNAc(3)Hex(5)Fuc(1)	1.39738	1.292584	0.181479	EGFR	0	0	1	0	Fucose
YADAGHVCHLCPNCTYOGTGP0LEOG PTNGPK_N4H4F1	N623_HexNAc(4)Hex(4)Fuc(1)	0.708429	0.29791	-0.42514	EGFR	0	0	1	0	Fucose

Supplemental Table 4a

UniProt	Gene Symbol	Protein Name
Q15054	POLD3	POLD3; DNA polymerase delta 3, accessory subunit
P39748	FEN1	FEN1; flap structure-specific endonuclease 1
P18858	LIG1	LIG1; DNA ligase 1
P49736	MCM2	MCM2; minichromosome maintenance complex component 2
P25205	MCM3	MCM3; minichromosome maintenance complex component 3
P33991	MCM4	MCM4; minichromosome maintenance complex component 4
P33992	MCM5	MCM5; minichromosome maintenance complex component 5
Q14566	MCM6	MCM6; minichromosome maintenance complex component 6
P33993	MCM7	MCM7; minichromosome maintenance complex component 7
P12004	PCNA	PCNA; proliferating cell nuclear antigen
Q9NRF9	POLE3	7 POLE3; DNA polymerase epsilon 3, accessory subunit
P09884	POLA1	POLA1; DNA polymerase alpha 1, catalytic subunit
P49005	POLD2	POLD2; DNA polymerase delta 2, accessory subunit
P49642	PRIM1	PRIM1; DNA primase subunit 1
P49643	PRIM2	PRIM2; DNA primase subunit 2
Q9NR33	POLE4	5 POLE4; DNA polymerase epsilon 4, accessory subunit
P35251	RFC1	RFC1; replication factor C subunit 1
P35250	RFC2	RFC2; replication factor C subunit 2
P40938	RFC3	RFC3; replication factor C subunit 3
P35249	RFC4	RFC4; replication factor C subunit 4
P40937	RFC5	RFC5; replication factor C subunit 5

Supplemental Table 4b

UniProt	Gene Symbol	Protein Name
P24941	CDK2	cyclin dependent kinase 2
Q9UM13	ANAPC10	anaphase promoting complex subunit 10
O14757	CHEK1	checkpoint kinase 1
Q9Y5N6	ORC6	origin recognition complex subunit 6
Q13257	MAD2L1	mitotic arrest deficient 2 like 1
P49736	MCM2	minichromosome maintenance complex component 2
P25205	MCM3	minichromosome maintenance complex component 3
P33991	MCM4	minichromosome maintenance complex component 4
P33992	MCM5	minichromosome maintenance complex component 5
Q14566	MCM6	minichromosome maintenance complex component 6
P33993	MCM7	minichromosome maintenance complex component 7
P12004	PCNA	proliferating cell nuclear antigen
P53350	PLK1	polo like kinase 1
Q13535	ATR	ATR serine/threonine kinase
P06400	RB1	RB transcriptional corepressor 1
P24385	CCND1	cyclin D1
O60566	BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B
P33981	TTK	TTK protein kinase
O00311	CDC7	cell division cycle 7
P20248	CCNA2	cyclin A2
P14635	CCNB1	cyclin B1
Q99640	PKMYT1	protein kinase, membrane associated tyrosine/threonine 1
O95067	CCNB2	cyclin B2
O95997	PTTG1	PTTG1 regulator of sister chromatid separation, securin
P06493	CDK1	cyclin dependent kinase 1
Q12834	CDC20	cell division cycle 20
P30307	CDC25C	cell division cycle 25C
P30260	CDC27	cell division cycle 27