Supporting Information

SI Materials and Methods

Chemicals. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (Cat. #C4706, Sigma Aldrich), Hydroxylamine solution (NH_2OH) (Cat. #467804, Sigma Aldrich), Hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) (Cat. #159417, Sigma Aldrich), Disuccinimidyl glutarate (DSG) (Cat. #20593, Thermo scientific), N-Ethylmaleimide (NEM) (Cat. #04259, Sigma Aldrich), Methoxypolyethylene glycol maleimide (PEG-maleimide or mPEG) (Cat. #63187, Sigma Aldrich), Iodoacetamide (IAA) (Cat. #I1149, Sigma Aldrich), Alkyne Palmitoyl-Coenzyme A (Cat. #15968, Cayman), Myristoyl Coenzyme A lithium (Cat. #M4414, Sigma Aldrich), Palmitoyl Coenzyme A lithium (Cat. #P9716, Sigma Aldrich), Stearoyl Coenzyme A lithium (Cat. #S0802, Sigma Aldrich), Palmitoleoyl Coenzyme A lithium (Cat. #P6775, Sigma Aldrich), Oleoyl Coenzyme A lithium (Cat. #O1012, Sigma Aldrich), Palmostatin B (Cat. #178501, Millipore), Triacsin C (Cat. #SC-200574, Santa Cruz).

Cloning, Cell culture, Transfection and Infection. Full-length human RFX3 cDNA was obtained from DF/HCC DNA Resource Core, and was subcloned into pCMV-3xFlag, pEGFP-N1 and pBABE vectors. All the mutants were generated by site-directed mutagenesis using the QuickChange II Site-Directed Mutagenesis kit (Agilent) following manufacturer's instructions. HEK293A, Phoenix, NIH3T3 cells were obtained from ATCC, and cultured in high-glucose Dulbecco's modified Eagles media (DMEM) (Life technologies) with 10% (v/v) fetal bovine serum (FBS) (Thermo/Hyclone, Waltham, MA) and 50 μg/mL penicillin/streptomycin. MIN6 cells were cultured in high-glucose DMEM with 15% FBS, 15 mM HEPES, 79 μM 2-mercaptoethanol, and 50 μg/mL penicillin/streptomycin. Expression constructs were transfected into cells using jetPRIME transfection reagent (Polyplus transfection) or polyethylenimine (PEI). Retrovirus was prepared using Phoenix cells. After infection, cells were selected for 2 weeks under puromycin before experiments. None of cell lines used in this paper are listed in the database of commonly misidentified cell lines maintained by ICLAC. All cell lines are free of mycoplasma contamination.

Generation of RFX3 Knockout Stable Cell Lines. Two different sgRNAs-targeting mouse RFX3 (KO1: CTATTTGATCGGCAATTCAA and KO2: GGGATTCAGATGGGCGTCAC) were cloned into pLentiCRISPR v2 (lentiCRISPR v2 was a gift from Feng Zhang's lab). Viruses were produced following the published protocols from Zhang lab. The empty vector pLentiCRISPR v2 was taken as control. Briefly, a 10 cm dish of 80% confluent HEK293T cells was transfected using 10 μg of the transfer plasmid, 2.5 μg pVSVG, 7.5 μg psPAX2, 500 μL of jetPRIME buffer, and 25 μL of jetPRIME transfection reagent. Media was changed after incubation overnight. After 48 hours, viral supernatants were filtered through a 0.45 μm low protein binding membrane (Millipore) and used immediately. Transduction was performed in 6-well plate and selected with 1 μg/mL puromycin for 2 weeks. RFX3 knockout efficiency was analyzed by western blot. The mixed cell populations were used for the experiments.

Generation of RFX3 expression stable cell lines. Flag-tagged RFX3 or the C544S mutant was cloned into pBABE-hygromycin retroviral vector. Retroviruses were produced in Pheonix packaging cell lines. Transduction of NIH3T3 or MIN6 stable cells (RFX3 knockout or vector control) was performed in 6-well plate and selected under 100 μg/mL hygromycin B for 3 days. Flag-RFX3 expression was analyzed by western blot.

Analysis of Fatty Acylation Sites on RFX3. The Flag-tagged RFX3 mutants were generated by site-directed mutagenesis using the QuikChange kit (Agilent). HEK293A cells were transfected with pCMV-3xFlag vector control, wild-type (WT) Flag-RFX3 construct, or mutant (C429S, C431S, C542S, C544S and C739S) construct. Cells were treated for 4 hours with Alk-C16. The cells lysates were reacted with azide-biotin and subjected to SDS-PAGE. Streptavidin blotting using streptavidin-HRP (1:5000, Life technologies) was performed to analyze fatty acylated RFX3.

LC-MS/MS Analysis of RFX3 Fatty Acylation. Flag-RFX3 was transfected into HEK293A cells. At 48 hours post-transfection, the cells were washed three times with cold PBS and lysed with lysis buffer containing 50 mM Tris·HCl, pH7.4, 150 mM NaCl, 1% Triton X-100, 1 mM PMSF and 1 mM EDTA, 1 x EDTA-free cOmplete protease inhibitor cocktail (Roche), 1 x phosphatase inhibitor cocktail, 10 mM TCEP and 50 mM NEM. After

centrifuging at maximum speed for 20 minutes, the supernatant was incubated for overnight with pre-washed anti-Flag magnetic beads (Cat. #M8823, Sigma Aldrich) in the cold room. After washing three times with buffer A (50mM Tris·HCl, 150 mM NaCl, 5mM EDTA, 0.2% Triton X-100, pH7.4) the beads were treated with buffer A or 1 M NH_2OH (pH 7.4) in buffer A for 2 hours at room temperature (RT). Then the beads were washed with buffer B (50 mM Tris·HCl, 150 mM NaCl, 0.2% Triton X-100, pH7.4) for three times, followed by incubation with 10 mM IAA in buffer B in the dark at RT for 1 hour. After washing with buffer B, RFX3 was eluted by 0.1 M Glycine-HCl, pH3.0 followed by neutralization with 1 M Tris·HCl, pH8.0 or eluted by 2 x SDS sample buffer at 95 °C for 10 minutes. The SDS sample buffer elution was separated by SDS-PAGE and subjected to in-gel digestion as described previously (1). Peptides from two sample (NH_2OH) and two control (without NH_2OH) were labeled with TMT10-plex reagents (channels 126, 127n, 127c, and 128n) (2) and subjected to LC-MS2-(SPS)MS3 analysis (3, 4) on an Orbitrap Lumos mass spectrometer (Thermo Scientific) essentially as described previously (5) but without any additional off-line fractionation.

Quantitative RT-PCR. Total RNA was extracted from NIH3T3 or MIN6 cells by using TRIzol reagent (Invitrogen). cDNA was produced by using High Capacity cDNA Reverse Transcription Kit (Cat. #4368814, Thermo Scientific). Quantitative RT-PCR analysis was performed by using LightCycler 480 SYBR Green I Master in the LightCycler 480 instrument (Roche). The mouse primers were selected from PrimerBank database (http://pga.mgh.harvard.edu/primerbank/). The sequences (5' –> 3') are listed as follows: Dync2li1 (Forward: AATCGCAAAAGCTGAGGTAGAG, Reverse: GTTGTCTTTCCCCCGTTTTTACT); Dnaic1 (Forward: ATGCCCTCGAAACAGATTCGT, Reverse: GCTTTGGATTGGGACCATTCAT); Dnali1 (Forward: GGTGAGCCGGAATACAGAGAA, Reverse: TGTTTGGTAGGATCTGGGACA); Gck (Forward: TGAGCCGGATGCAGAAGGA, Reverse: GCAACATCTTTACACTGGCCT); Gapdh (Forward: AGGTCGGTGTGAACGGATTTG, Reverse: TGTAGACCATGTAGTTGAGGTCA). The final concentration of primers is 0.5 μM. Melting point analysis revealed only one PCR product was amplified. Results were normalized with respect to the internal control

and expressed as percentage of respective controls.

Western Blot Analysis. Cells were lysed with RIPA buffer supplemented with protease inhibitors (Roche) and phosphatase inhibitors (Roche). Lysates were denatured by heating for 5 minutes at 95 °C and loaded onto 4-12% Bis-Tris polyacrylamide gel. NuPAGE MES running buffer (Invitrogen) was used for the SDS-PAGE. The proteins were subsequently transferred to polyvinylidene fluoride membranes (Millipore). The membranes were blocked and incubated with primary antibodies and secondary HRP-conjugated antibodies, and developed by exposure to film. Antibody and dilutions used in the studies: anti-FLAG M2 (Cat. #F1804, Sigma Aldrich, 1:5000), anti-GFP (Cat. #SC-9996, Santa Cruz, 1:2000), anti-RFX3 (Cat. #HPA035689, Sigma Aldrich, 1:200), anti-GAPDH (Cat. #2118S, Cell signaling, 1:1000), anti-β-actin (Cat. #ab6276, Abcam, 1:500), anti-c-Myc (Cat. #M4439, Sigma Aldrich, 1:1000).

Protein Crosslinking Assay. RFX3 WT and C544S mutant construct were transfected into the HEK293A cells in 6-well plate, respectively. After 36 hours post-transfection, cells treated with 1% formaldehyde (HCHO) as negative control or 1 mM DSG. After 10 minutes incubation in cold PBS, HCHO- and DSG-treated cells were quenched with 100 mM glycine pH 3.0 and 500 mM Tris·HCl, pH 8.0, respectively for an additional 15 minutes. Cells were lysed by adding 100 µl of RIPA buffer to each well. Protein complexes were analyzed by western blot.

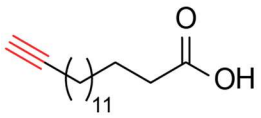
Co-immunoprecipitation Experiments. To test the dimerization of RFX3, Flag-RFX3 WT (or C544S mutant) and GFP-RFX3 (or C544S mutant) were co-transfected into HEK293A cells. ZDHHC7-interacting proteins were immunoprecipitated by anti-GFP antibody (Cat. #SC-9996, Santa Cruz), and Flag-RFX3 was detected in the complex by western blot using anti-FLAG M2 (Cat. #F1804, Sigma Aldrich, 1:5000).

Immunofluorescent Staining and Confocal Microscopy. Cells were seeded on glass cover slips in 6-well plate. After 48 hours incubation, cells were washed 3 times with PBS and fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. Then fixed cells were washed with PBS and permeabilized with 0.1% Triton X-100 at room temperature for 5 min. After blocking, coverslips were incubated for one hour with primary antibody

and then washed 3 times with 1% Triton X-100. Alexa Fluor-conjugated secondary antibodies (Life technologies) were used at a 1:5000 dilution in blocking buffer. After washing 3 times with PBS, the cover slips were mounted with VECTASHIELD mounting medium (Cat. #H-1200, Vector laboratories). Antibody and dilutions used in the studies: anti-FLAG M2 (Cat. #F1804, Sigma Aldrich, 1:1000), anti-acetylated tubulin (Cat. #T7451, Sigma Aldrich, 1:100), anti-acetylated tubulin (Cat. #5335S, Cell signaling, 1:100). More than 200 cells are quantified in at least three independent experiments.

Hedgehog Signaling Analysis. NIH3T3 cells were seeded on 6-well plate at 4 x 10^5/well. When the cells reached confluency after 48 hours incubation, they were stimulated with 100 nM Hedgehog agonist Ag1.5 or equal volume of DMSO (control) in fresh medium for overnight. The stimulated cells were collected for RT-PCR to analyze the expression of Hedgehog target genes including Gli1 and Ptch1.

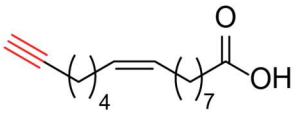
Statistics. Statistical analysis was carried out by means of one-way ANOVA with Bonferroni comparison test or by two-tailed Student's *t* test using GraphPad Prism software, version 5.0a (GraphPad).



2

Alk-C16

15-hexadecynoic acid



5

Alk-C16:1

cis-9-hexadecen-15-ynoic acid

Mass spectral peptide counts

Protein \ Probe	DMSO	**2**	**5**
RFX3	0	5/4	2/4
RFX1	0	5/7	4/6

Fig. S1. S-fatty acylation of RFX1 and RFX3. Identification of RFX fatty acylation by chemical reporters and mass spectrometry. HEK293A or MIN6 cells were incubated overnight with DMSO or 50 μM Clickable probes (**2** or **5**). Cell lysates were reacted with azide-biotin for enrichment of labeled proteins with streptavidin beads and identified by mass spectrometry. The peptide spectral counts were shown in the table.

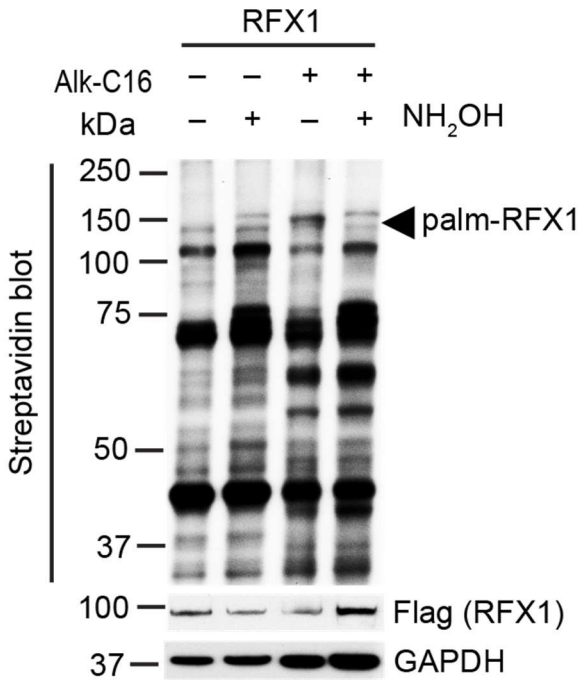


Fig. S2. RFX1 is S-fatty acylated. HEK293A cells were transfected with Flag-RFX1. At 36 hours post transfection, cells were labeled for 4 hours with Alk-C16. Click reaction was performed to analyze SCRIB palmitoylation status. Cell lysates were reacted with azide-biotin. Half of the sample was treated with hydroxylamine and analyzed by streptavidin blot. Hydroxylamine treatment led to decreased fatty acylation level of RFX1.

544

RFX3	ANVQEQASWVCQCDDNMVQRLETDF	-556
RFX1	ANVQEQASWVCRCEDRVVQRLEQDF	-811
RFX2	ANVQEQASWVCQCEESVVQRLEQDF	-571

Fig. S3. Sequence alignment of RFX3, RFX1 and RFX2 by ClustalW. The numbers stand for the specific position of the amino acids in different RFX family members. The highly conserved fatty acylation site of RFX3 (Cys544) is indicated. Cys796 in RFX1 and Cys559 in RFX2 are potential fatty acylation sites.

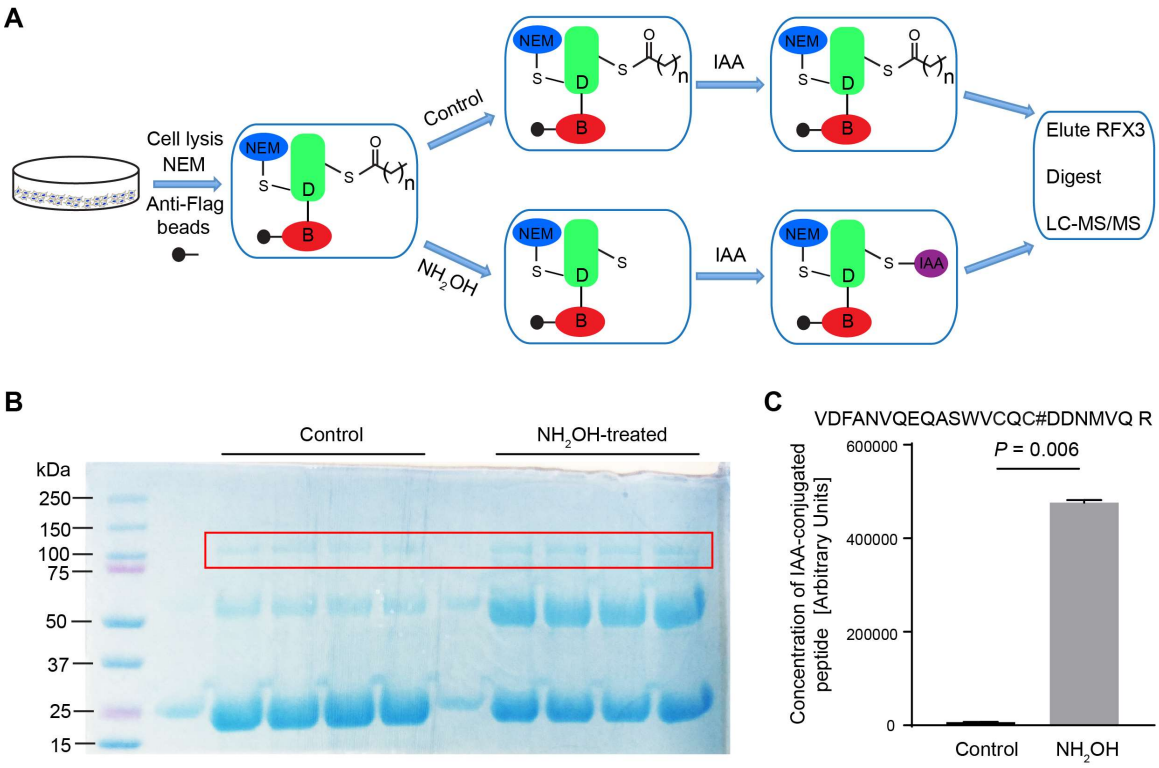


Fig. S4. LC-MS/MS analysis of RFX3 fatty acylation. (*A*) Scheme of LC-MS/MS analysis. Flag-tagged RFX3 was overexpressed in HEK293A cells and immunoprecipitated using anti-Flag beads, followed by sequential treatment with N- NEM, with/without NH_2OH and IAA. RFX3 was eluted from beads and separated by SDS-PAGE. The gel in red box was cut and subjected to in-gel digestion before LC-MS/MS analysis. (*B*) Coomassie staining of protein gels. Control and NH_2OH-treated samples were shown. (*C*) Quantification of the IAA-conjugated peptide. C#, IAA modified Cys544. NH_2OH treatment significantly increased the abundance of IAA-conjugated peptide. Values represent the average ± SEM, $P = 0.006$ (Student's *t* test, 2-tailed, unequal variance).

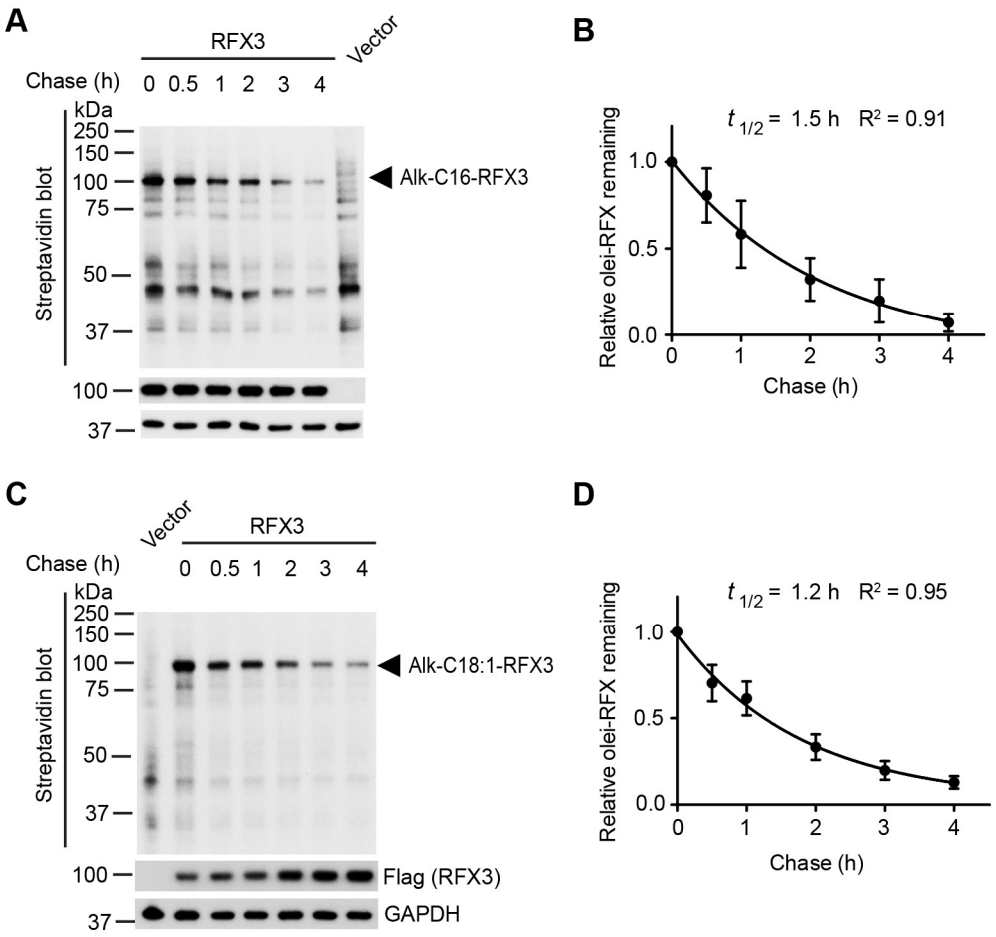


Fig. S5. Pulse-chase analysis shows dynamic S-fatty acylation of RFX3. (*A*) Pulse-chase experiments show dynamic S-palmitoylation of RFX3. (*B*) Determination of the half-life for the palmitic acid turnover on RFX3 from pulse-chase experiments. (*C*) Pulse-chase experiments show dynamic S-oleoylation of RFX3. (*D*) Determination of the half-life for the oleic acid turnover on RFX3 from pulse-chase experiments. Values represent the average ± SEM of three independent experiments.

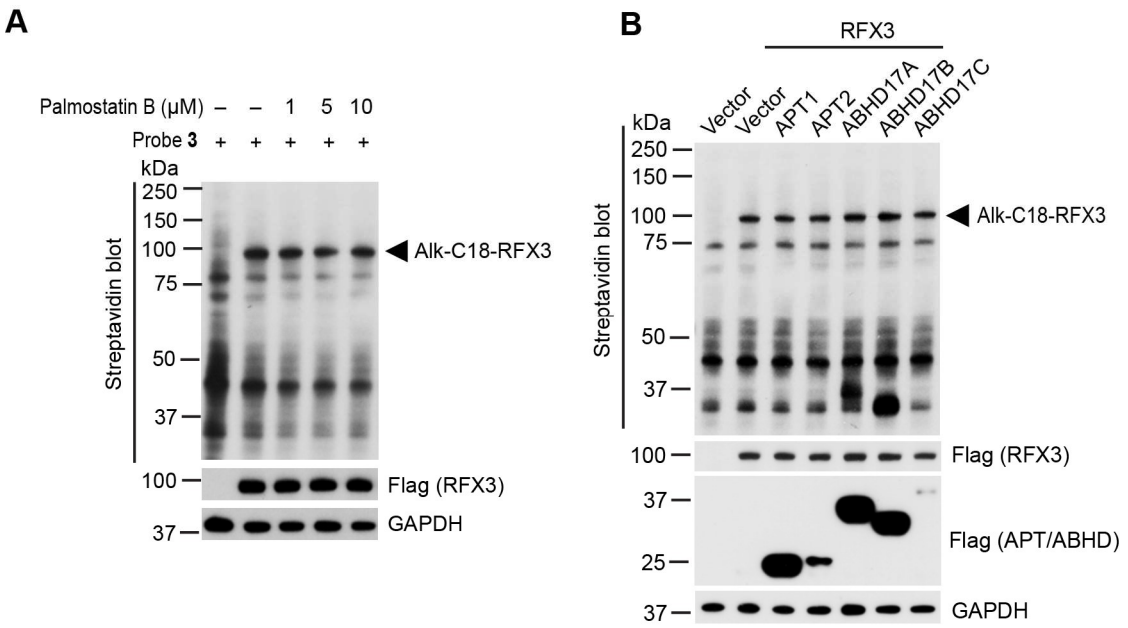


Fig. S6. Analysis of depalmitoylases involved in RFX3 defatty acylation in cells. (*A*) Palmostatin B shows little effect on RFX3 fatty acylation in HEK293A cells. At 24 hours post-transfection of Flag-RFX3, the transfected HEK293A cells were treated with DMSO control or Palmostatin B (1 μM, 5 μM and 10 μM) for 24 hours. Cell labeling followed by Click reaction experiments to analyze the effect of Palmostatin B on RFX3 fatty acylation. (*B*) Depalmitoylases APT1/2 and ABHD17 are not involved in RFX3 defatty acylation. HEK293A cells were transfected with Flag-RFX3 and split into 6-well plate at 8 hours post-transfection. After 16 hours incubation, Flag-tagged APT1/2, ABHD17A/B/C or vector control was transfected into the HEK293A cells. At 36 hours post-transfection, cells were labeled for overnight with Alk-C18 probe. Cells lysates were reacted with azide-biotin and subjected to SDS-PAGE. Streptavidin-conjugated horseradish peroxidase was used to detect RFX3 fatty acylation. Western blotting was performed to show the expression level of Flag-RFX3, Flag-tagged APT1/2 and ABHD17A/B/C.

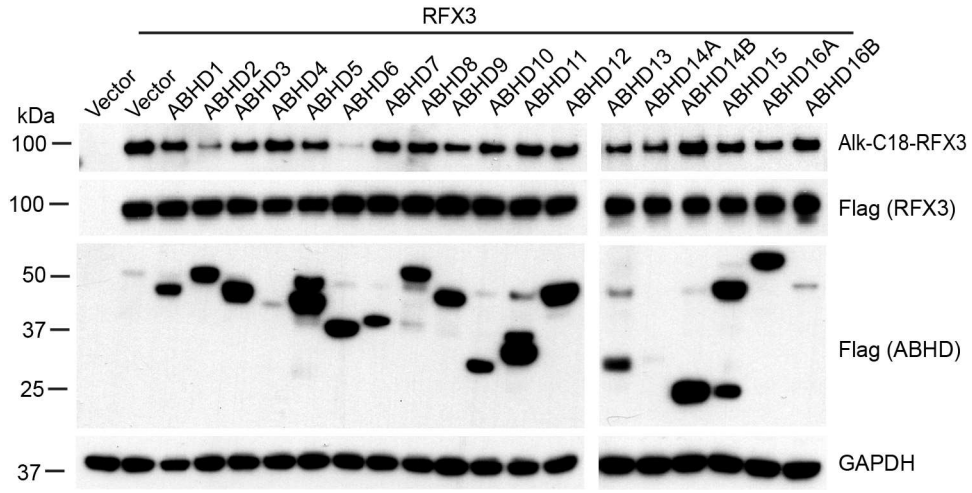


Fig. S7. Screening of potential ABHDs regulating RFX3 defatty acylation. Flag-tagged RFX3 and Flag-tagged ABHDs or vector control were co-transfected into the HEK293A cells. At 36 hours post-transfection, cells were labeled for overnight with Alk-C18 probe. Cells lysates were reacted with azide-biotin followed by streptavidin pulldown and SDS-PAGE. Western blotting results show the fatty acylated RFX3 (Alk-C18-RFX3), expression level of Flag-RFX3 and Flag-ABHDs. ABHD2 and ABHD6 are potential candidates regulating RFX3 defatty acylation.

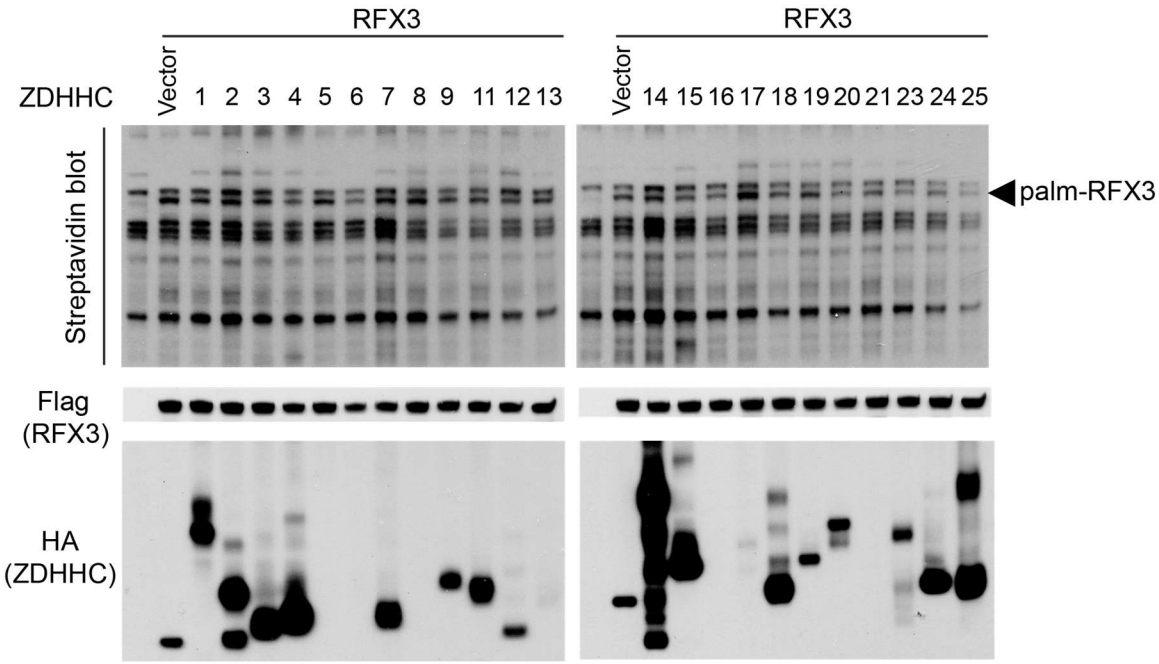


Fig. S8. Screening of potential ZDHHC-PATs regulating RFX3 fatty acylation. HEK293A cells were transfected with Flag-RFX3 and 23 HA-tagged ZDHHCs or HA-tagged GST as control in 6-well plate. After 36 hours, cells were labeled for 4 hours with Alk-C18 probe. Cells lysates were reacted with azide-biotin and subjected to SDS-PAGE. Streptavidin-conjugated horseradish peroxidase was used to detect RFX3 fatty acylation. Western blots for RFX3 total protein and HA-tagged ZDHHC proteins are shown.

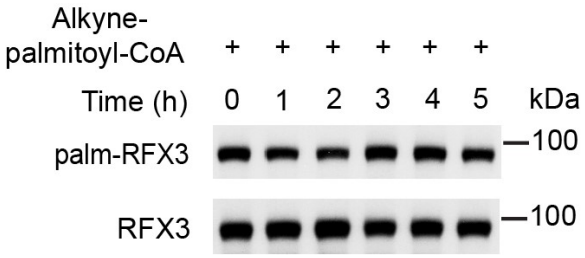


Fig. S9. Analysis of RFX3 autodefatty acylation *in vitro*. After 2 hours incubation of RFX3 (0.1 μM) with Alkyne-palmitoyl-CoA (5 μM), the excess Alkyne-palmitoyl-CoA was removed by buffer exchange. The fatty acylation level of RFX3 at indicated time points was determined by Click reaction followed by streptavidin blot. The reaction buffer contains 50 mM Tris·HCl, pH 7.5, 150 mM NaCl, and 1X cOmplete EDTA-free protease inhibitor.

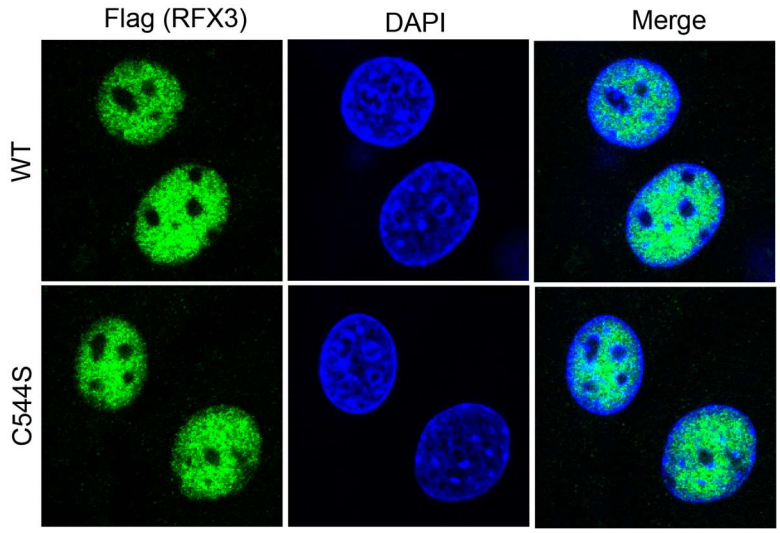


Fig. S10. Localization of RFX3 WT and C544S mutant in NIH3T3 cells. Established RFX3 knockout NIH3T3 cells were re-introduced RFX3 WT and C544S mutant. Immunofluorescence assay was performed to analyze the localization of Flag-tagged RFX3. Cells were stained with anti-Flag antibody (green) and DAPI (blue). Images were collected by using a confocal microscope.

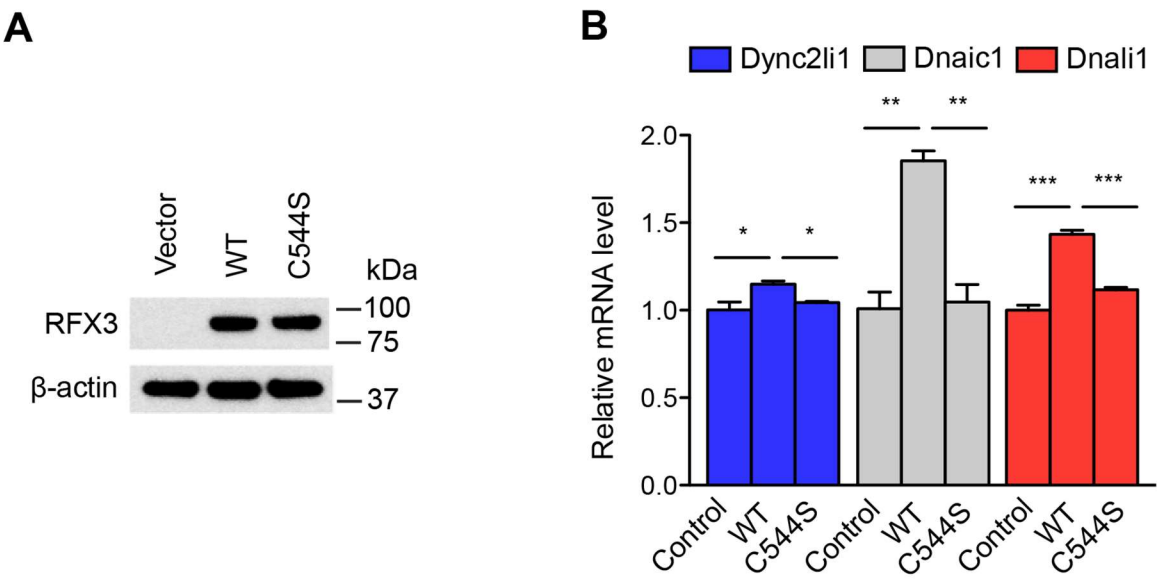


Fig. S11. RFX3 fatty acylation regulates ciliary gene expression.

(*A*) Western blot showing comparable expression level of RFX3 in NIH3T3 stable cell lines expressing WT RFX3 and the C544S mutant. (*B*) RT-PCR results showing expression of ciliary genes in the RFX3 stable cell lines. Values represent the average ± SEM of three experiments. Different from control at **P*< 0.05, ***P*< 0.01, ****P*< 0.001 (Student's *t* test).

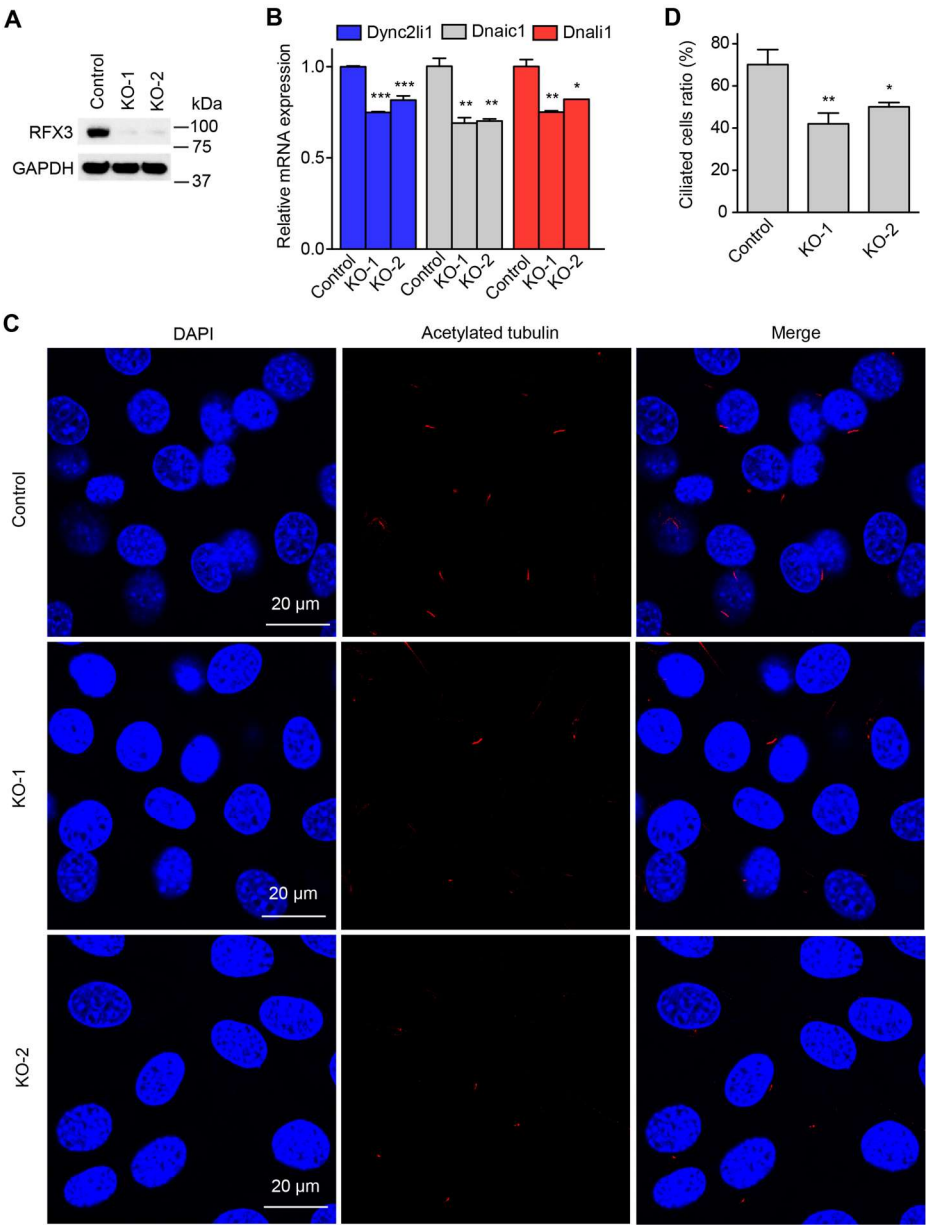


Fig. S12. Knockout of RFX3 in NIH3T3 cells leads to decreased ciliary gene expression and cilia formation. (*A*) Western blot showing RFX3 knockout efficiency in NIH3T3 cells. (*B*) RT-PCR results showing expression of ciliary genes in the RFX3 knockout stable cell lines. (*C*) Representative images of cilia in the RFX3 knockout stable cell lines. Cells were stained with anti-acetylated tubulin antibody (red), and DAPI (blue). Scale bars represent 20 μm. (*D*) Quantification of the percentage of ciliated cells in different stable cell lines. At least 200 cells were analyzed for each cell line in three independent experiments. Values represent the average ± SEM of three experiments. Different from control at **P*< 0.05, ***P*< 0.01, ****P*< 0.001 (Student's *t* test).

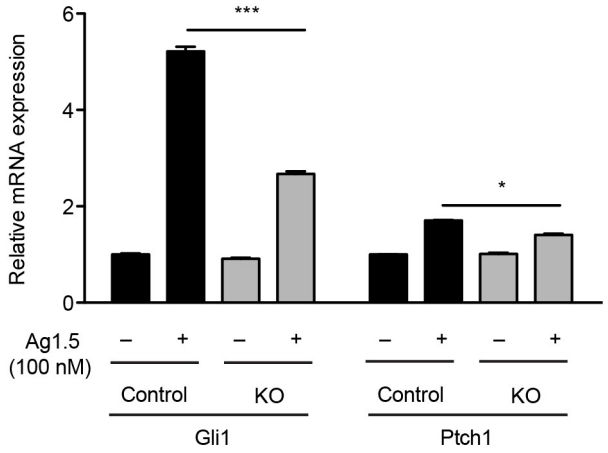


Fig. S13. Knockout of RFX3 in NIH3T3 cells leads to decreased Hedgehog signaling. After treatment with 100 nM of Hedgehog agonist Ag1.5 for overnight, RT-PCR assay was performed to evaluate Hedgehog target genes (Gli1 and Ptch1) in the established NIH3T3 stable cell lines. Compared with vector control, the expression levels of Gli and Ptch1 are significantly decreased in the RFX3 knockout cells. Values represent the average ± SEM of three experiments. Different from control at **P*< 0.05, ****P*< 0.001 (Student's *t* test).

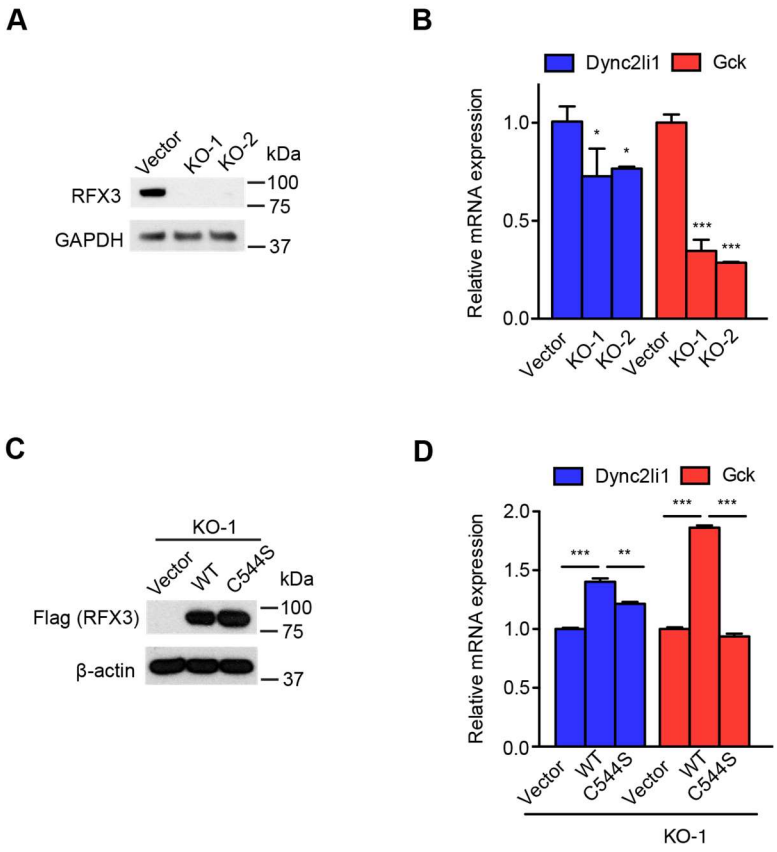


Fig. S14. Fatty acylation of RFX3 regulates glucokinase gene Gck expression in pancreatic β-cell line MIN6. (*A*) MIN6 cell lines with stable knockout of RFX3 (KO-1 and KO-2) were generated by CRISPR/Cas9 genome-editing system, and the knockout efficiencies were confirmed by western blot analysis Western blot. (*B*) Using the established stable cell lines, RT-PCR was performed assay to analyze the known RFX3 target genes including the ciliary gene Dync2li1 as a positive control and glucokinase gene Gck. In pancreatic β-cells, Gck encodes a glucokinase protein which is known as the important pancreatic β-cell glucose sensor. (*C*) After re-introducing WT RFX3 and the fatty acylation-deficient mutant C544S into the RFX3-knockout cell line KO-1, western blot showing comparable expression level of WT RFX3 and the C544S mutant in the established stable cells. (*D*) RT-PCR results shows that re-introducing of WT RFX3, but not fatty acylation-deficient mutant C544S, significantly increases the expression levels of Gck and Dync2li1. Values represent the average ± SEM of three experiments. Different from control at **P*< 0.05, ***P*< 0.01, ****P*< 0.001 (Student's *t* test).

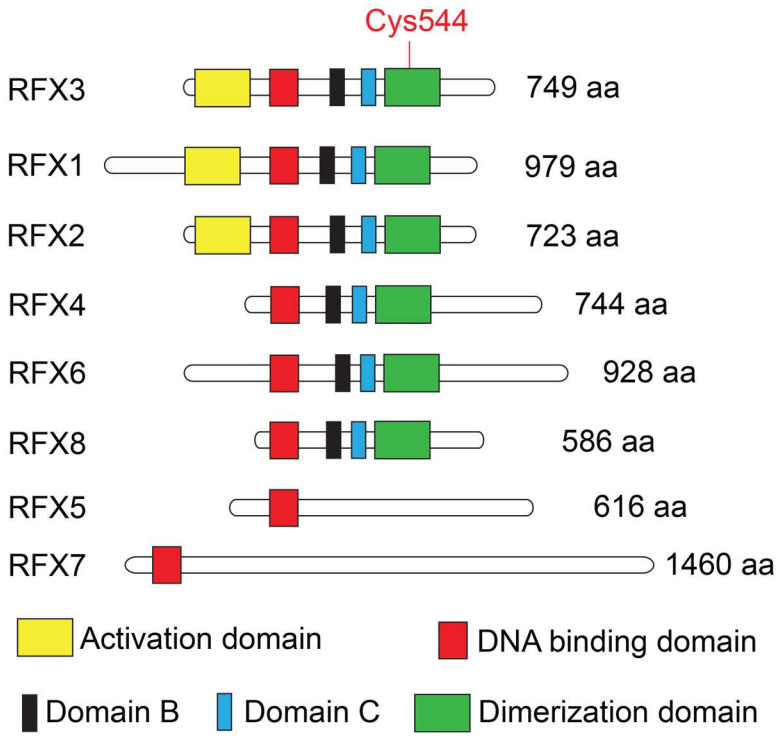


Fig. S15. Schematic representation of human RFX family proteins and fatty acylation site of RFX3. Eight members of human RFX proteins are shown. Conserved RFX protein domains and fatty acylation site of RFX3 are highlighted. Yellow, Activation domain; Red, DNA binding domain (DBD); Black, Domain B; Blue, Domain C; Green, Dimerization domain (DD). Cys544 is the fatty acylation site of RFX3.

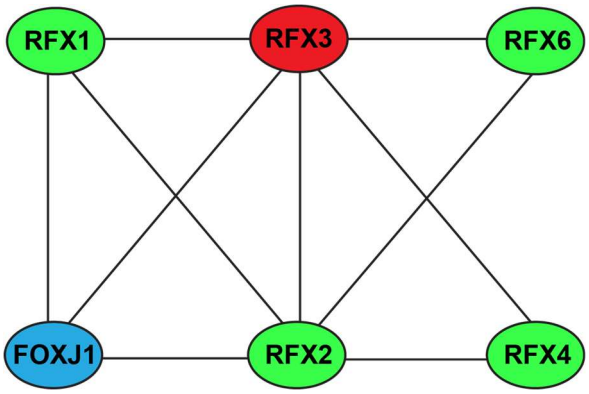


Fig. S16. RFX protein interaction network. RFX family proteins, including RFX1-4 and RFX6 can interact with each other and form homo- or hetero-dimers. RFX proteins can also physically interact with other partner proteins such as FOXJ1, which is a critical transcriptional factor for the formation of motile cilila. RFX2, RFX3, RFX4 and FOXJ1 have been shown as key transcriptional regulators of ciliogenesis, including both immotile and motile cilia. Here, ovals represent proteins and lines indicate protein-protein interactions. The protein interactions have been validated by co-immunoprecipitation (co-IP) or yeast two-hybrid screening (6-9).

Table S1. IAA modified cysteine peptides

Peptide sequence [1]	IAA modified site(s) [2]	Modscore(s) [3]	Ion charge	Xcorr [4]	DCn [4]	Ppm [5]	Normalized intensities [6]				Log2 ratio	
							ctrl_1	ctrl_2	treated_1	treated_2	avg treated/ avg ctrl	p-value (treated, ctrl) [7]
R.VDFANVQEQASWVC#QCDDNM^VQR.L	542	4.085	3	4.508	0.116	-0.1	23899.93758	21622.97183	433451.6519	486177.0056	4.336386754	0.038016901
R.VDFANVQEQASWVCQC#DDNM^VQR.L	544	4.54	3	7.761	0.088	0.83	6968.126299	6584.447005	480168.412	471858.86	6.134364187	0.005534715
R.VDFANVQEQASWVCQC#DDNM^VQR.L	544	4.54	3	7.39	0.11	1.51	6968.126299	6584.447005	480168.412	471858.86	6.134364187	0.005534715
R.VDFANVQEQASWVCQC#DDNM^VQR.L	544	4.54	3	5.952	0.107	0.13	6968.126299	6584.447005	480168.412	471858.86	6.134364187	0.005534715
R.VDFANVQEQASWVCQC#DDNMVQR.L	544	4.54	3	6.16	0.032	1.8	22914.82473	28705.75754	471943.9765	435610.8308	4.135966450	0.023557664
R.VDFANVQEQASWVC#QC#DDNMVQR.L	542, 544	1000, 1000	3	6.822	0.667	1.12	20515.5912	20366.71209	457474.7131	464636.8024	4.495392849	0.005154442
R.VDFANVQEQASWVC#QC#DDNMVQR.L	542, 544	1000, 1000	3	3.972	0.517	0.27	20515.5912	20366.71209	457474.7131	464636.8024	4.495392849	0.005154442
R.QC#SATGNTYTAV.-	739	1000	2	2.242	0.386	-0.01	110688.8884	99948.42244	372972.6582	354918.4569	1.788961647	0.004343952

[1]. Only peptides from RFX3 were considered for the analysis; NEM was selected as static modification so that a NEM modified cysteine residue is shown without symbol; # shows an IAA modification; ^ indicates oxidation of a methionine residue.

[2]. Position of the modified cysteine on RFX3

[3]. The modscore states the likelihood of the modification being annotated to the correct cysteine in the peptide sequence (10). A modscore larger than 13 reflects a p-value of smaller than 0.05 regarding an incorrect annotation, and a modscore of 19 reflects a p-value of smaller than 0.01.

[4]. XCorr and dCN are the two core-score of the SEQUEST algorithm used to annotate the acquired spectra

[5]. The deviation of the measured from the expected monoisotopic peptide mass.

[6]. Intensities were summed across all redundant peptides and normalized based on the intensities of all non-cysteine containing RFX3 peptides

[7]. p-values were calculated using the student's *t* test, two-tailed, unequal variance.

References

1. Haas W*, et al.* (2006) Optimization and use of peptide mass measurement accuracy in shotgun proteomics. *Molecular & cellular proteomics : MCP* 5(7):1326-1337.
2. McAlister GC*, et al.* (2012) Increasing the multiplexing capacity of TMTs using reporter ion isotopologues with isobaric masses. *Analytical chemistry* 84(17):7469-7478.
3. Ting L, Rad R, Gygi SP, & Haas W (2011) MS3 eliminates ratio distortion in isobaric multiplexed quantitative proteomics. *Nature methods* 8(11):937-940.
4. McAlister GC*, et al.* (2014) MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes. *Analytical chemistry* 86(14):7150-7158.
5. Lapek JD, Jr.*, et al.* (2017) Detection of dysregulated protein-association networks by high-throughput proteomics predicts cancer vulnerabilities. *Nat Biotechnol* 35(10):983-989.
6. Morotomi-Yano K*, et al.* (2002) Human regulatory factor X 4 (RFX4) is a testis-specific dimeric DNA-binding protein that cooperates with other human RFX members. *The Journal of biological chemistry* 277(1):836-842.
7. Smith SB*, et al.* (2010) Rfx6 directs islet formation and insulin production in mice and humans. *Nature* 463(7282):775-780.
8. Didon L*, et al.* (2013) RFX3 modulation of FOXJ1 regulation of cilia genes in the human airway epithelium. *Respir Res* 14:70.
9. Rual JF*, et al.* (2005) Towards a proteome-scale map of the human protein-protein interaction network. *Nature* 437(7062):1173-1178.
10. Beausoleil SA, Villen J, Gerber SA, Rush J, & Gygi SP (2006) A probability-based approach for high-throughput protein phosphorylation analysis and site localization. *Nat Biotechnol* 24(10):1285-1292.