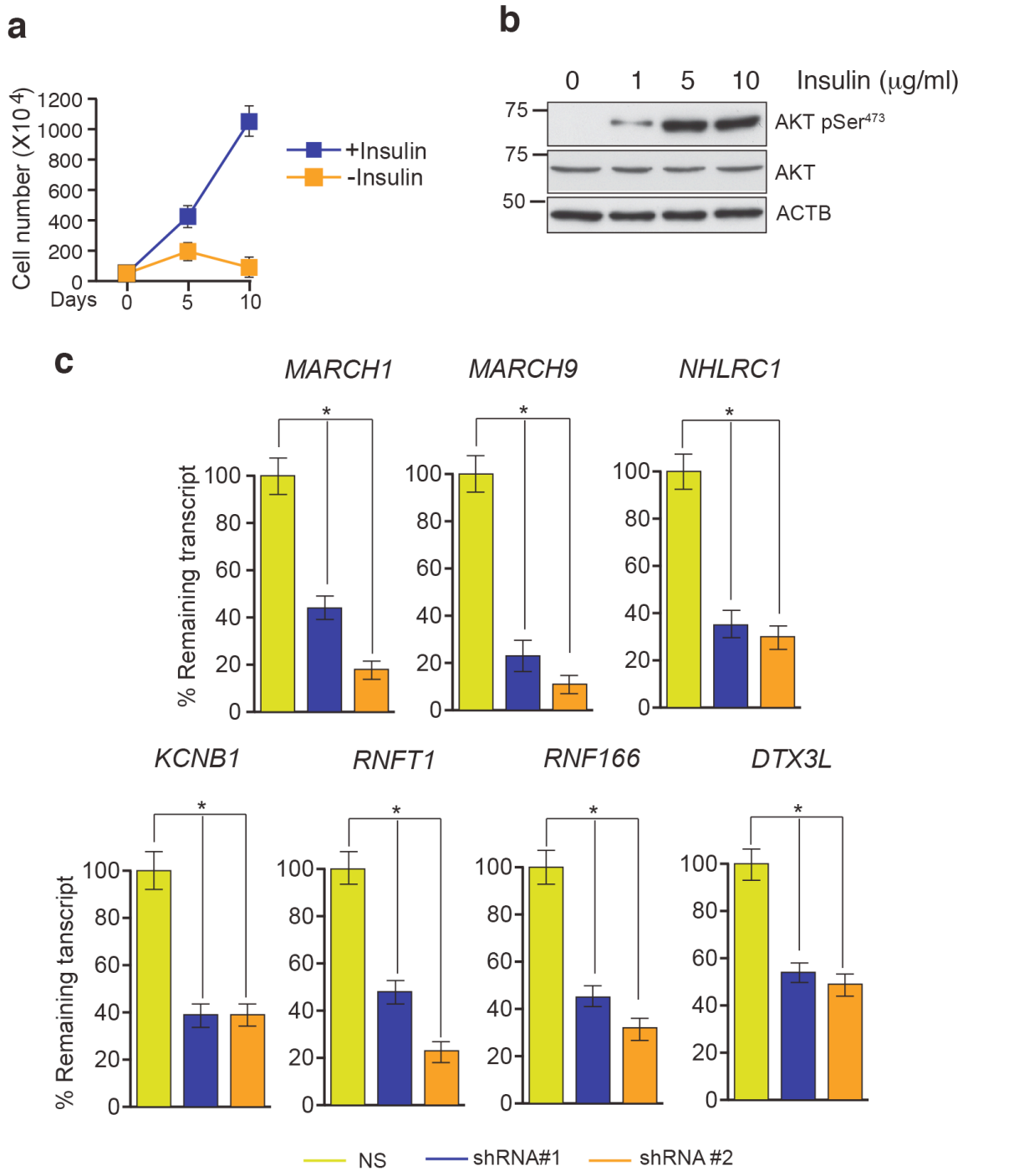
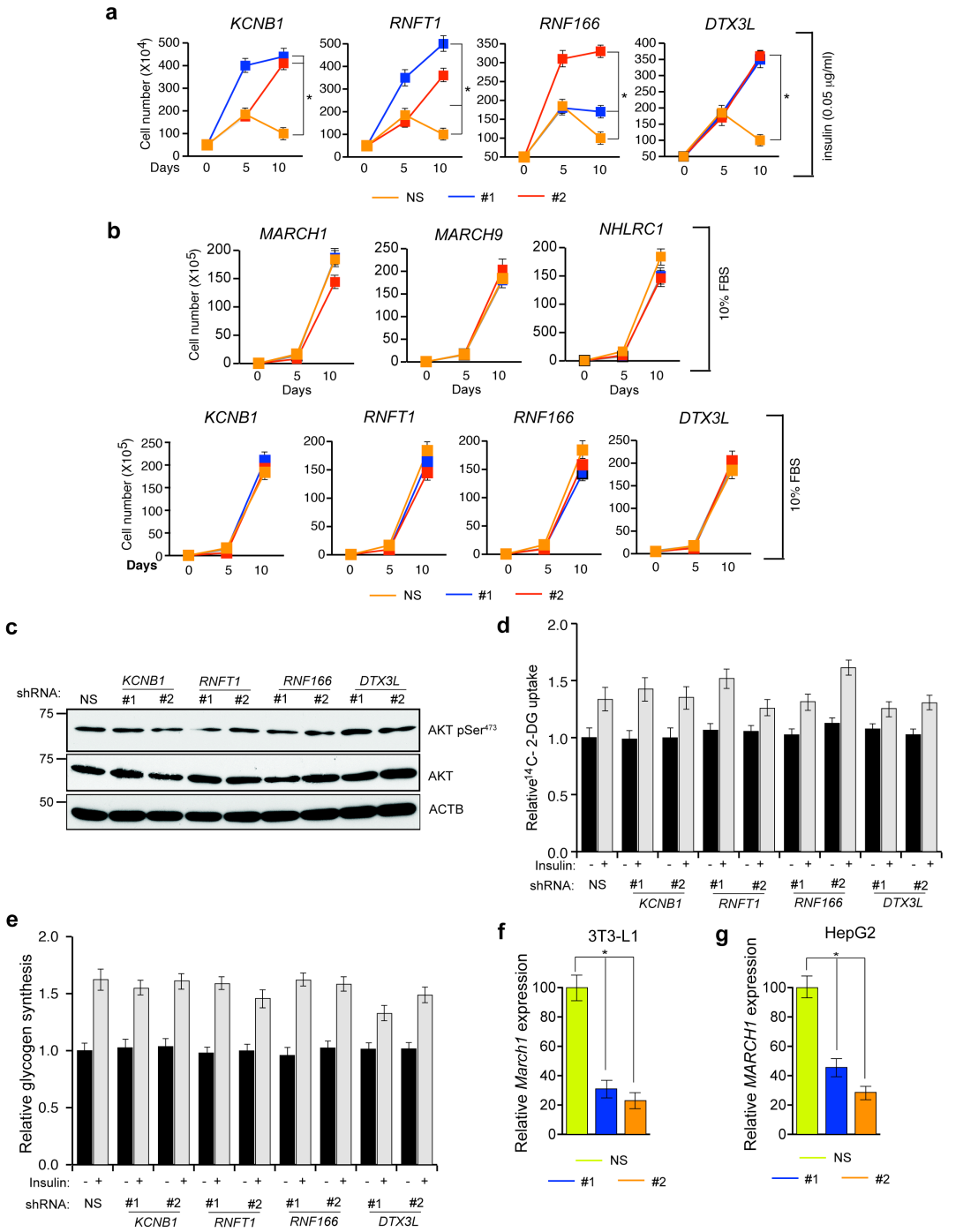
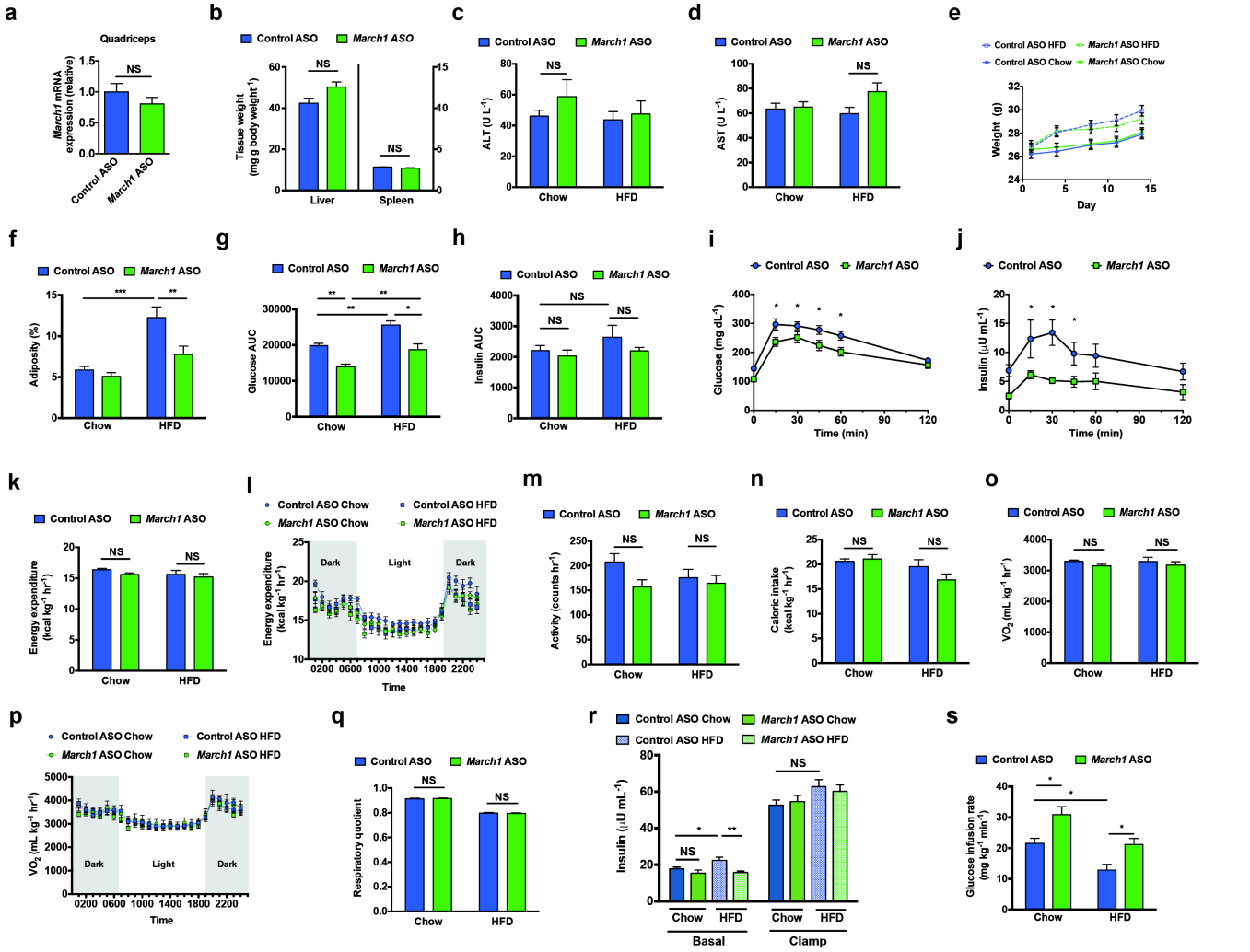


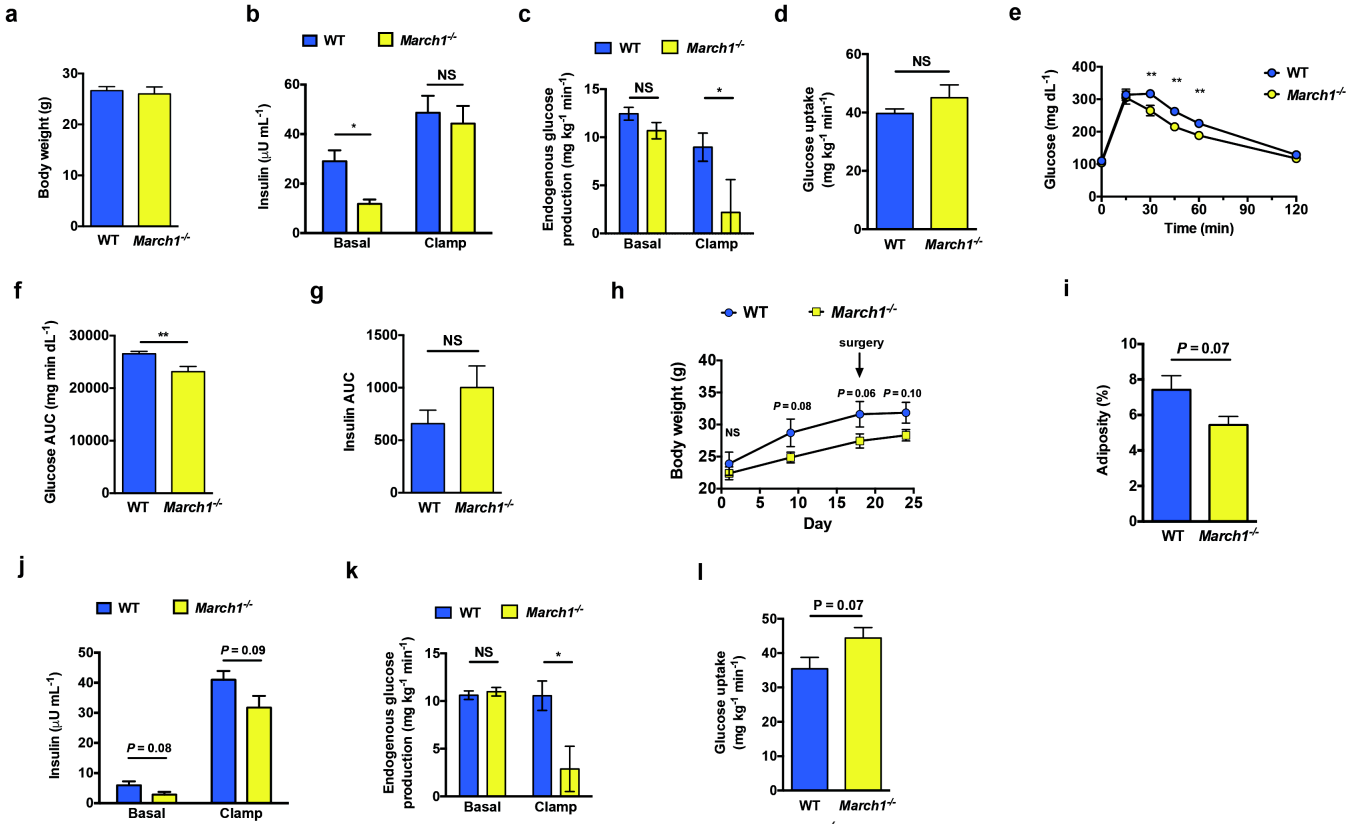
Supplementary Figure 1. HeLa cell survival, insulin signaling, and knockdown efficiency of identified candidates. (a) Cell proliferation, calculated using Trypan blue exclusion (n=3), for HeLa cells grown in serum free DMEM containing growth factors without insulin or 5 μg/ml insulin at days 5 and 10. **(b)** Insulin stimulation of AKT Ser^{473} phosphorylation. HeLa cells were serum starved overnight and then stimulated with indicated concentrations of insulin for 30 mins. **(c)** cDNA from HeLa cells expressing indicated shRNAs was analyzed by qRT-PCR (n=3). Knockdown efficiency relative to non-specific (NS) shRNA is shown. * compares NS shRNAs against indicated shRNAs. Data are mean ± SEM. In all panels, *P<0.05, comparisons by t-test.



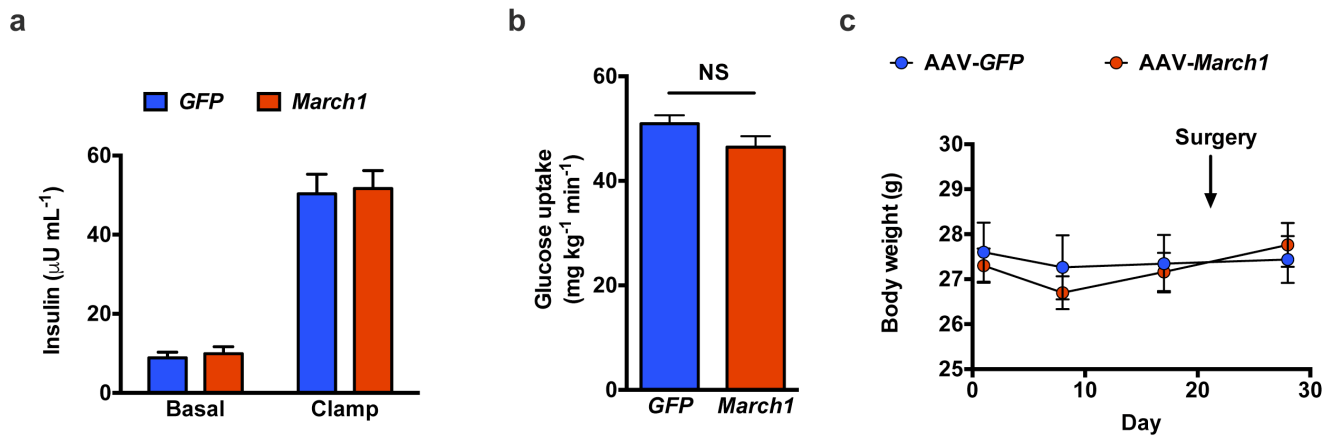
Supplementary Figure 2. Validation of candidates identified from the large-scale RNAi screen. (a) Relative cell number (n=3), calculated using Trypan blue exclusion, for HeLa cells expressing indicated shRNAs and grown in low-insulin medium at day 5 and 10. * compares NS and *MARCH1* shRNAs at day 10**. (b)** Relative cell number (n=3), calculated using Trypan blue exclusion, for HeLa cells expressing indicated shRNAs and grown in 10% serum-supplemented medium at days 5 and 10. **(c)** Immunoblot analysis of AKT Ser^{473} phosphorylation in HeLa cells expressing the indicated shRNAs. **(d)** Relative ^{14}C-2-deoxyglucose (2-DG) uptake (n=4) in HeLa cells expressing the indicated shRNAs. **(e)** Relative glycogen synthesis (n=3) in HeLa cells expressing the indicated shRNAs. **(f)** 3T3-L1 cells expressing *March1* or non-specific (NS) shRNA were analyzed for knockdown efficiency using qRT-PCR (n=3). **(g)** HepG2 cells expressing *MARCH1* or non-specific (NS) shRNA were analyzed for knockdown efficiency using qRT-PCR (n=3). Data are mean ± SEM. In all panels, *P<0.05, comparisons by *t*-test.



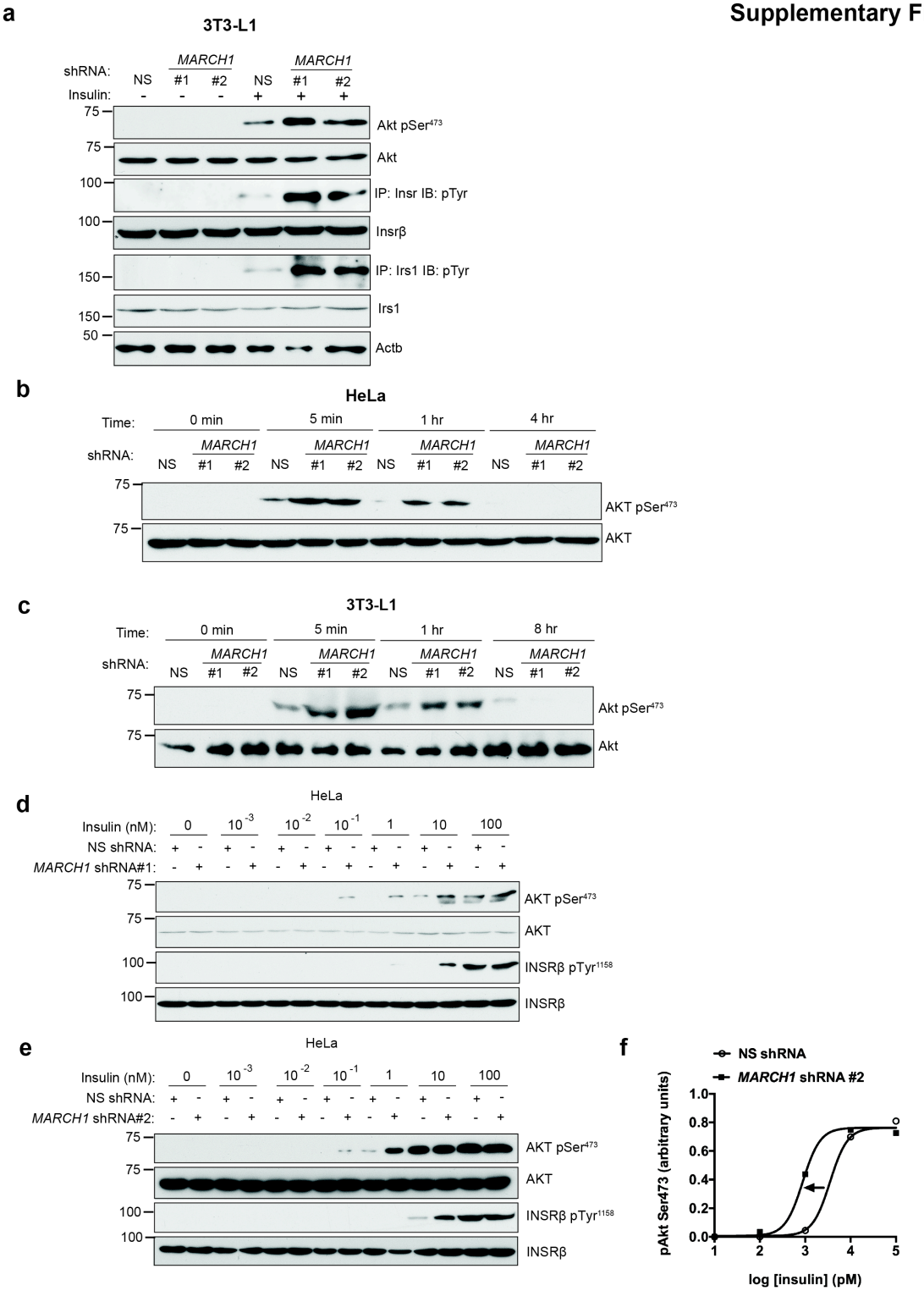
Supplementary Figure 3. Studies of male mice treated with *March1* ASO. (**a-h**) Mice were treated for two weeks with ASO and fed regular chow or HFD as indicated. **(a)** *March1* mRNA expression in quadriceps muscle. **(b)** Liver and spleen wet weight. **(c)** Plasma alanine transaminase (ALT) activity. **(d)** Plasma aspartate transaminase (AST) activity. **(e)** Body weight during ASO treatment. **(f)** Adiposity after ASO treatment measured by ^{1}H NMR. **(g)** Plasma glucose area under the curve during intraperitoneal glucose tolerance tests (ipGTTs) for data plotted in **Fig. 3b.** **(h)** Plasma insulin area under the curve during ipGTTs for data plotted in **Fig. 3c.** (**i-j**) Mice were treated with ASO for four weeks and fed regular chow. **(i)** Plasma glucose excursions during ipGTTs. **(j)** Plasma insulin excursions during ipGTTs. **(k-r)** Mice were treated with ASO for two weeks and fed chow or HFD as indicated before metabolic cage studies. **(k-l)** Whole-body energy expenditure. **(m)** Locomotor activity. **(n)** Caloric intake (**o-p)** Whole-body oxygen consumption. **(q)** Respiratory quotient. **(r-s)** Mice were treated with ASO for two weeks and fed regular chow or HFD as indicated before hyperinsulinemic-euglycemic clamp studies (see also **Fig. 3d-h**). **(r)** Plasma insulin levels before and during the clamp. **(s)** Mean glucose infusion rates during the steady-state period of the clamp for data plotted in **Fig. 3d**. Data are mean ± SEM. In all panels, *P<0.05, **P<0.005, ***P < 0.0005. In **(a-c)**, n = 6-7 mice per group; comparisons by two-way ANOVA. In **(e)**, n = 12-19 mice per group; comparisons by two-way ANOVA. In **(f)**, n = 18-23 mice per group; comparisons by two-way ANOVA. In **(g-h)**, n = 5-8 mice per group; comparisons by two-way ANOVA. In **(i-j)**, n = 8-9 mice per group; comparisons by two-tailed unpaired t-test. In **(k-q)**, n = 15-16 mice per group (regular chow) and 6-7 mice per group (HFD); comparisons by two-way ANOVA. In **(r-s)**, n = 9-11 mice per group; comparisons by two-way ANOVA.



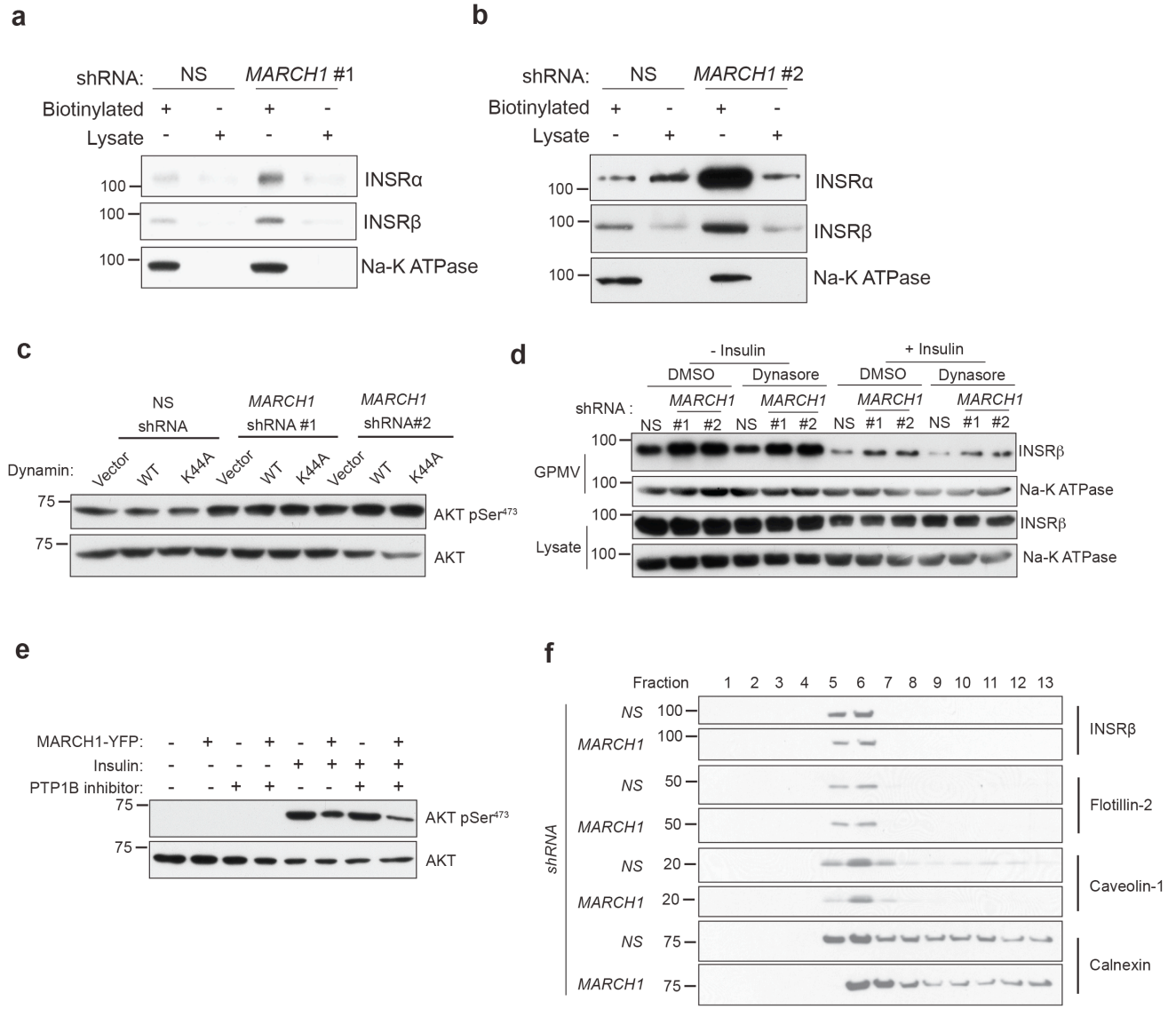
Supplementary Figure 4. Metabolic phenotyping of *March1*$^{-/-}$ mice. Littermate wild-type control mice were used in all studies of *March1*$^{-/-}$ mice. Different insulin infusion rates were used for studies of chow-fed and HFD-fed mice (2 mU kg^{-1} min^{-1} and 3 mU kg^{-1} min^{-1}, respectively), so data are not superimposed. **(a-d)** Hyperinsulinemic-euglycemic clamp studies were performed in chow-fed male mice (see also **Fig. 4a-c**). **(a)** Body weight. **(b)** Fasting and clamped plasma insulin concentrations. **(c)** Basal and clamped endogenous glucose production (EGP). **(d)** Whole-body glucose uptake during the steady-state period of the clamp. **(e-g)** Intraperitoneal glucose tolerance tests were performed in chow-fed female mice. **(e)** Plasma glucose excursions. **(f)** Plasma glucose area under the curve (AUC). **(g)** Plasma insulin AUC. **(h-l)** Hyperinsulinemic-euglycemic clamp studies were performed in HFD-fed mice (see also **Fig. 4d-f**). **(h)** Body weight during high-fat feeding. **(i)** Whole-body adiposity measured by ^{1}H NMR. **(j)** Fasting and clamped plasma insulin concentrations. **(k)** Basal and clamped EGP. **(l)** Whole-body glucose uptake during the steady-state period of the clamp. Data are mean ± SEM. In all panels, **P*<0.05, ***P*<0.005; comparisons by two-tailed unpaired *t*-test. In **(a-d)**, *n* = 10 WT and 5 KO mice. In **(e-g)**, *n* = 11 mice per group. In **(h-l)**, *n* = 5 mice per group.



Supplementary Figure 5. Hyperinsulinemic-euglycemic clamp studies in mice treated with AAV-*March1*. Male 12-week old C57BL/6J mice were injected intravenously with AAV four weeks before study and fed regular chow. Hyperinsulinemic-euglycemic clamps were performed (see also **Fig. 5**). **(a)** Plasma insulin levels before and during the clamp. **(b)** Whole-body glucose uptake during the steady-state period of the clamp. **(c)** Body weight during treatment. Data are mean ± SEM. In all panels, **P*<0.05. *n* = 7-9 mice per group; comparisons by *t*-test.

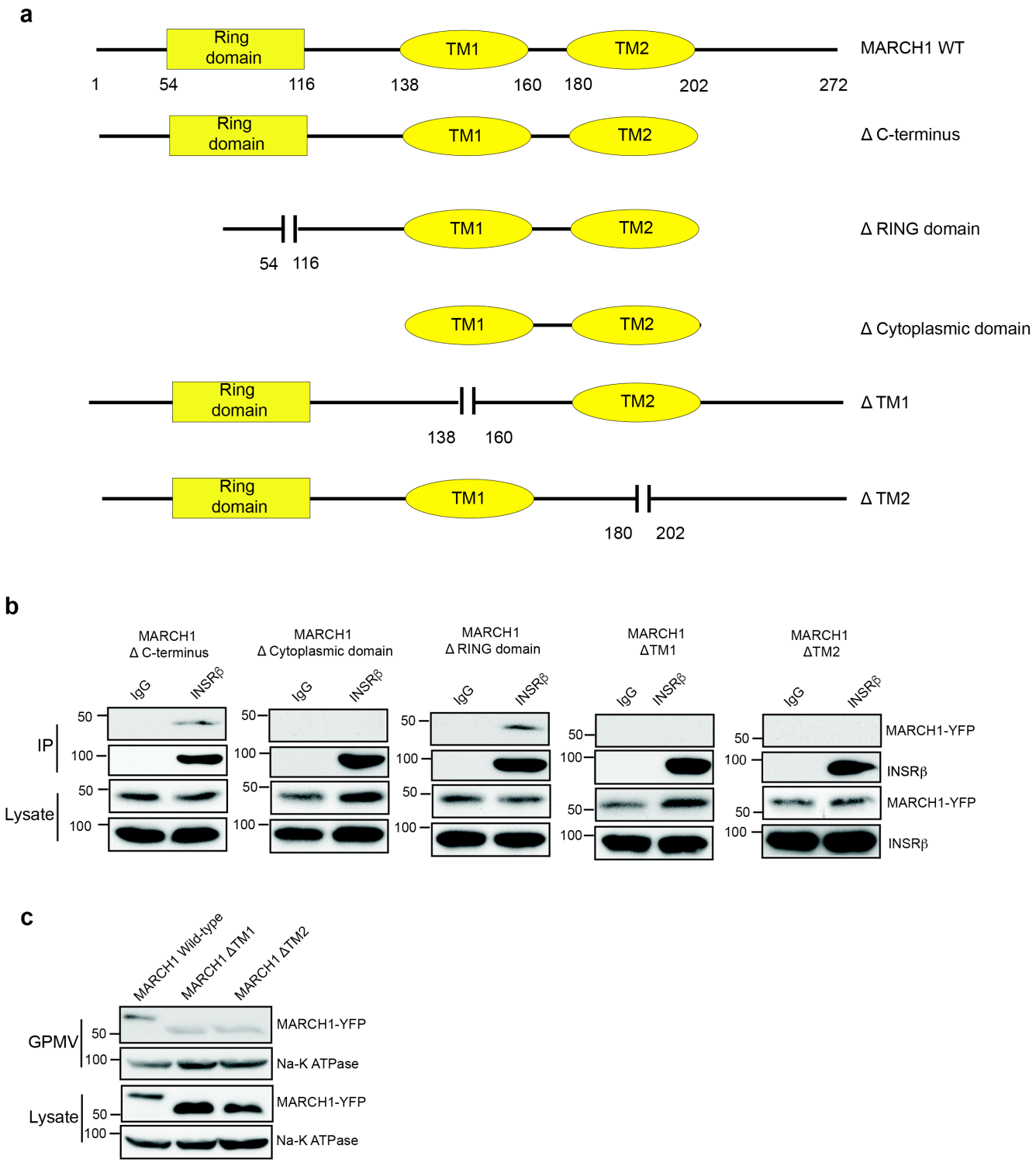


Supplementary Figure 6. Increased INSR signaling in cells with *MARCH1* knockdown. (a) Immunoblot analysis of insulin-stimulated INSR, IRS-1, and AKT activation in 3T3-L1 cells expressing the indicated shRNAs. **(b-c)** Insulin stimulation time course studies. HeLa cells **(b)** or 3T3-L1 cells **(c)** expressing the indicated shRNAs were serum starved overnight and stimulated with insulin (0.05 μg/mL) for the indicated duration. AKT Ser^{473} phosphorylation was assessed by immunoblotting. **(d-e)** Insulin dose-response studies. HeLa cells expressing the indicated shRNAs were serum starved overnight and stimulated with insulin at the indicated concentration for 30 min. AKT Ser^{473} and INSR Tyr^{1158} phosphorylation were assessed by immunoblotting. **(f).** Quantitation of the immunoblots in (**e**) fitted to insulin dose-response curves. See **Fig. 7c** for quantitation of the immunoblots in (**d**).

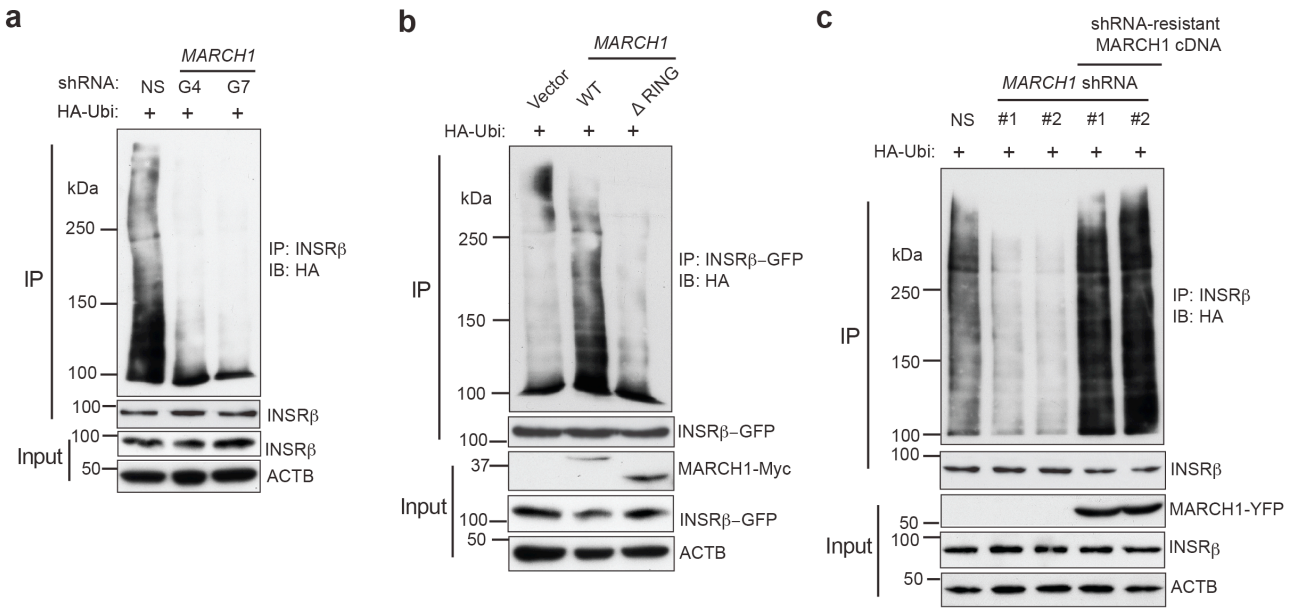


Supplementary Figure 7. MARCH1 regulates cell surface INSR expression without altering endocytosis, PTP1B activity, or membrane microdomain INSR content. (a-b) HeLa cells expressing the indicated shRNAs were serum starved and surface proteins were biotinylated. Avidin-isolated cell surface proteins and whole cell lysates were analyzed for the indicated proteins. See **Fig. 7d** for quantitation of multiple biotinylation experiments using *MARCH1* shRNA #1. **(c)** HeLa cells expressing indicated shRNAs were transfected with either empty vector (Vector), wild-type dynamin (WT) or endocytosis-defective K44A dynamin (K44A) and analyzed for AKT Ser^{473} phosphorylation. **(d)** HeLa cells expressing the indicated shRNAs and treated with or without the dynamin inhibitor Dynasore (20 μM) were harvested before or after 3 hr stimulation with 5μg/ml insulin. Immunoblot analysis of INSRβ in GPMVs or whole cell lysate was performed. Na-K ATPase is shown as a loading control. **(e)** HeLa cells were serum starved overnight and then stimulated with 0.05 μg/ml insulin. Samples were collected after 30 minute of insulin stimulation, either in the presence of DMSO or PTP1B inhibitor ($C_{26}H_{19}Br_2N_3O_7S_3$) (10 μM)**. (f)** Lipid rafts and caveolae were isolated by sucrose gradient fractionation from HeLa cells expressing the indicated shRNAs and were analyzed for marker proteins and INSRβ by immunoblot analysis.

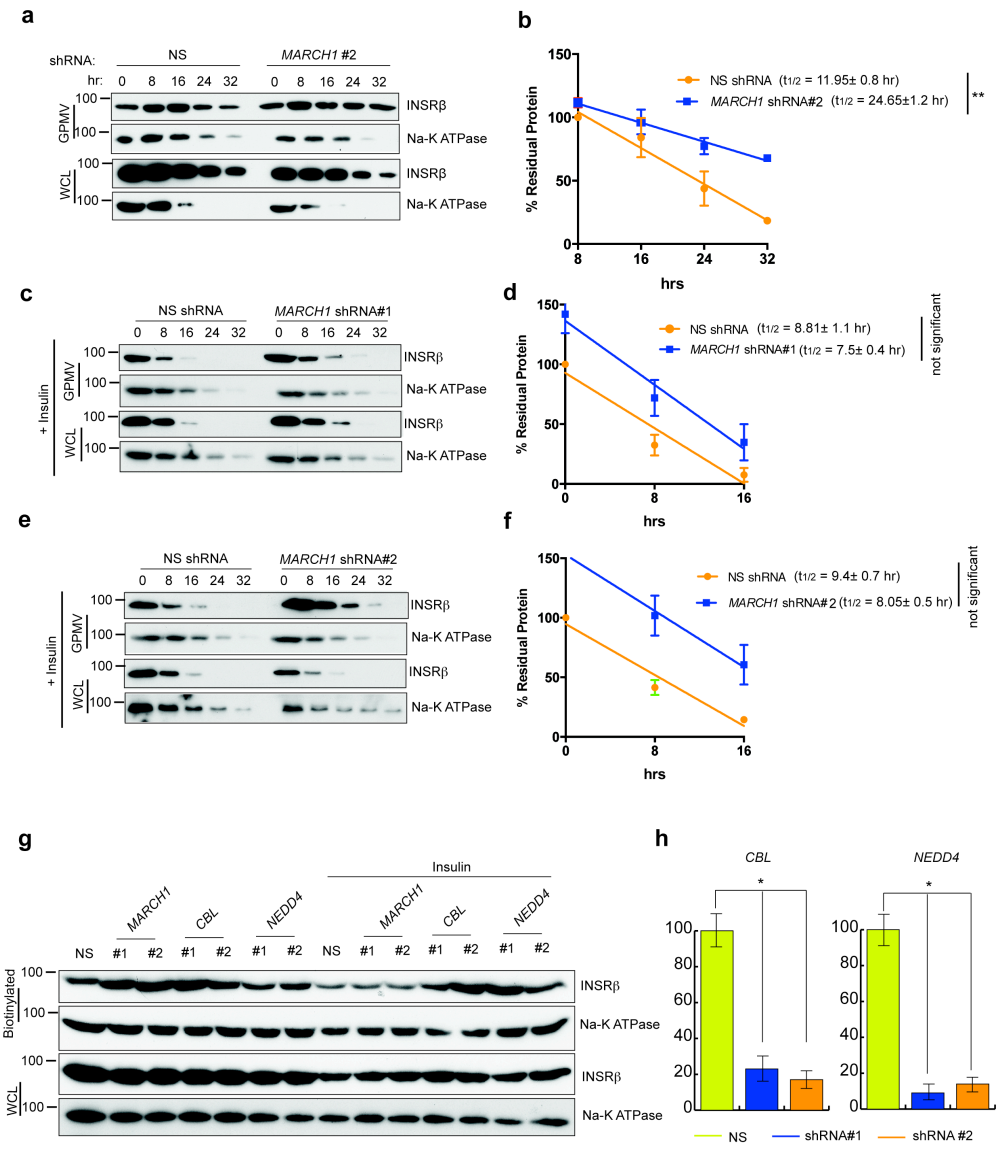
Supplementary Fig. 8



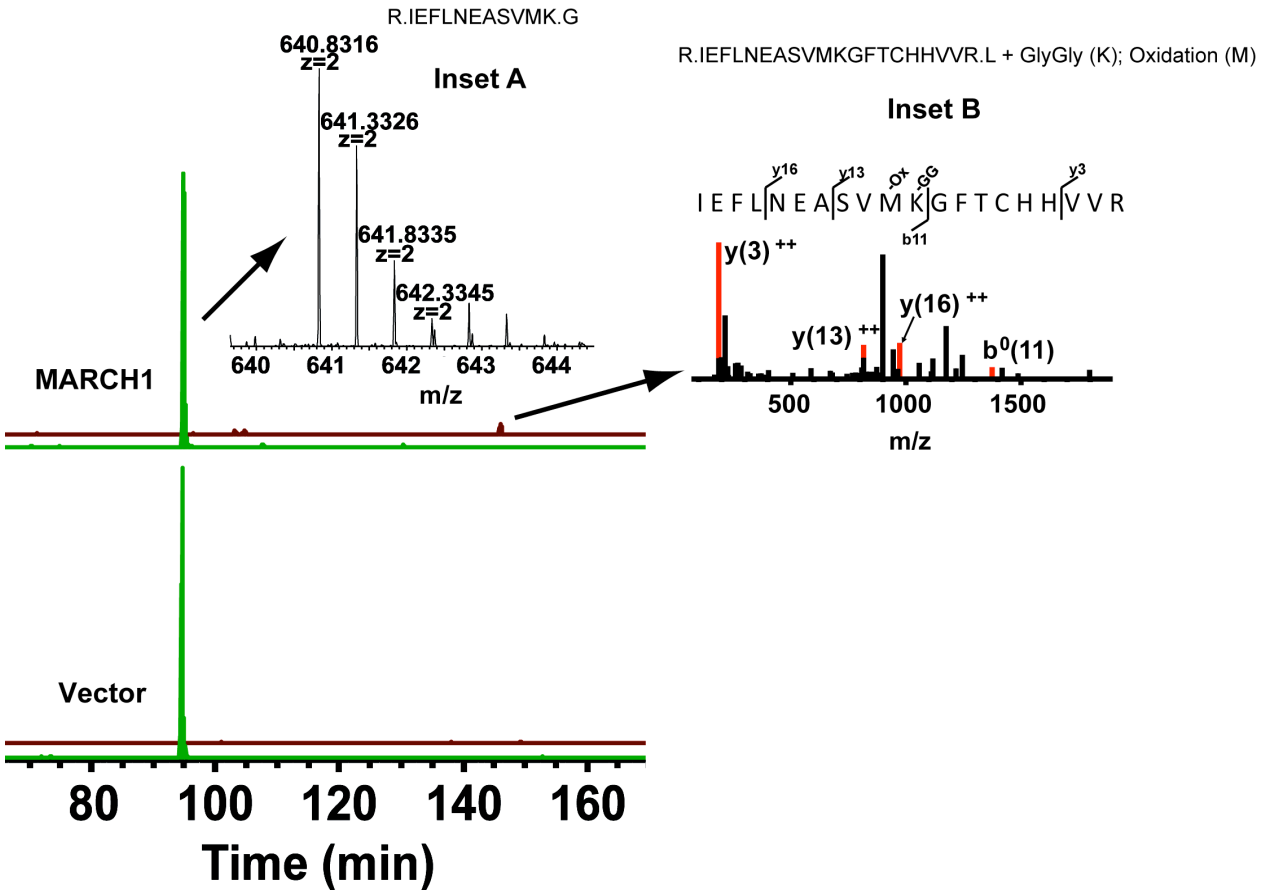
Supplementary Figure 8. (a) Schematic for the MARCH1 deletion mutants. **(b)** Indicated MARCH1 deletion mutants were transfected in HeLa cells along with INSRβ and immunoprecipitation was performed using either IgG or anti-INSRβ antibody. Both IP products and whole cell lysates were analyzed for indicated protein by immunoblotting. (**c**) Either wild type or indicated MARCH1 transmembrane deletion mutants were transfected in HeLa cells and GPMV and whole cell lysates were analyzed for indicated proteins by immunoblotting to assess MARCH1 localization.



Supplementary Figure 9. MARCH1 ubiquitinates INSR. (a) Immunoblot analysis of INSRβ ubiquitination. INSRβ immunoprecipitated from HeLa cells expressing NS or *MARCH1* shRNAs and HA-ubiquitin was immunoblotted for HA. Lysates were probed for the indicated proteins. **(b)** Immunoblot analysis of INSRβ polyubiquitination. INSRβ immunoprecipitated from HeLa cells expressing *MARCH1-Myc* or *MARCH1-ΔRING-Myc* and HA-ubiquitin was immunoblotted for HA. Lysates were probed for indicated proteins. **(c)** Immunoblot analysis of INSRβ polyubiquitination. INSRβ immunoprecipitated from HeLa cells expressing NS or *MARCH1* shRNA #1 or #2 with or without shRNA-resistant *MARCH1* cDNA was immunoblotted for HA-ubiquitin. Lysates were probed for indicated proteins.



Supplementary Figure 10. MARCH1 regulates surface INSRβ half-life through direct ubiquitination. (a) HeLa cells expressing NS or *MARCH1* shRNA#2 were serum starved overnight and treated with cycloheximide (100 μg/ml). GPMVs and whole cell lysates (WCL) were collected at indicated time points and analyzed for indicated proteins by immunoblotting. **(b)** Quantitation of the immunoblots (n=3) shown in (a). **(c)** HeLa cells expressing indicated shRNAs were serum starved overnight and treated with insulin (5 μg/ml) and cycloheximide (100 μg/ml). GPMVs and whole cell lysates (WCL) were collected at indicated time points and analyzed for indicated proteins by immunoblotting. **(d)** Quantitation of the immunoblots (n=3) shown in (c). **(e)** HeLa cells expressing indicated shRNAs were serum starved overnight and treated with insulin (5 μg/ml) and cycloheximide (100 μg/ml). GPMVs and whole cell lysates (WCL) were collected at indicated time points and analyzed for indicated proteins by immunoblotting. **(f)** Quantitation of the immunoblots (n=3) shown in (e). **(g)** Biotinylation assay. HeLa cells expressing the indicated shRNA were serum starved overnight and left untreated or insulin treated (5 μg/ml) for 3 hrs. The biotinylated fraction and whole cell lysates were analyzed for INSRβ and Na-K ATPase content. **(h)** HeLa cells expressing non-specific (NS) or *CBL* or *NEDD4* shRNA were analyzed for knockdown efficiency using qRT-PCR (n=3). Data are mean ± SEM. **P*<0.05 and ***P*<0.05, comparisons by *t*-test.



Supplementary Figure 11. Mass spectrometry analysis of INSRβ in HeLa cells transfected with vector or MARCH1. Extracted ion chromatograms are shown for the non-modified (green) and modified (red) INSRβ peptide derived from vector- (lower) and *MARCH1*- (upper) transfected HeLa cells. Inset A shows the averaged mass spectrum corresponding to the non-modified peptide. Inset B indicates the corresponding MS/MS fragmentation of the modified peptide at m/z 816.4086 with assigned b- and y- fragment ions. Above the MS/MS spectrum is the modified peptide sequence indicating the location of the identified modified lysine residue. Note that methionine is oxidized.

Figure 1c

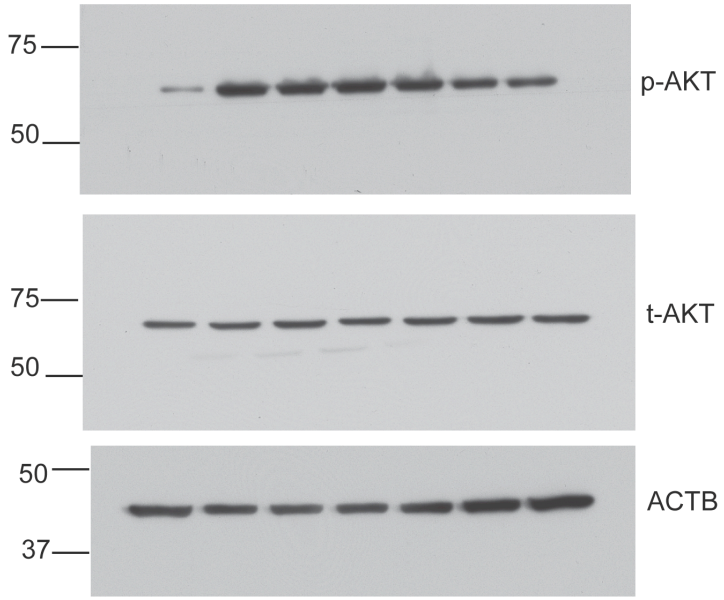


Figure 2c

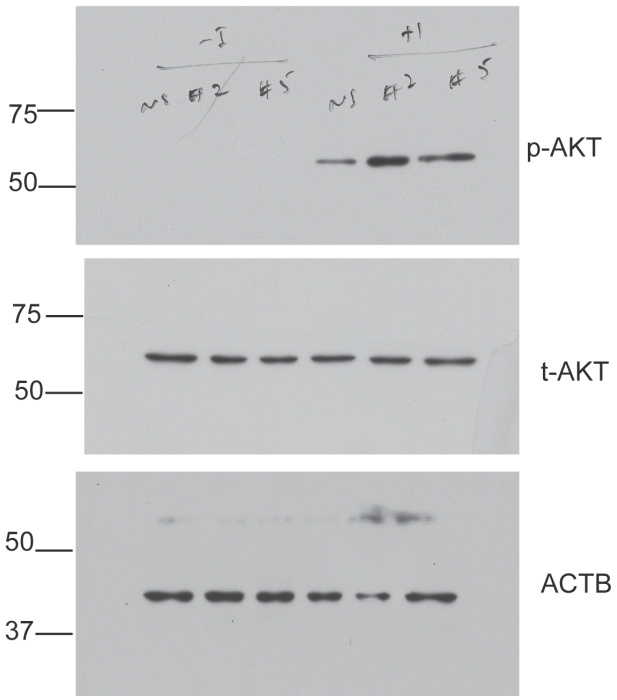


Figure 2f

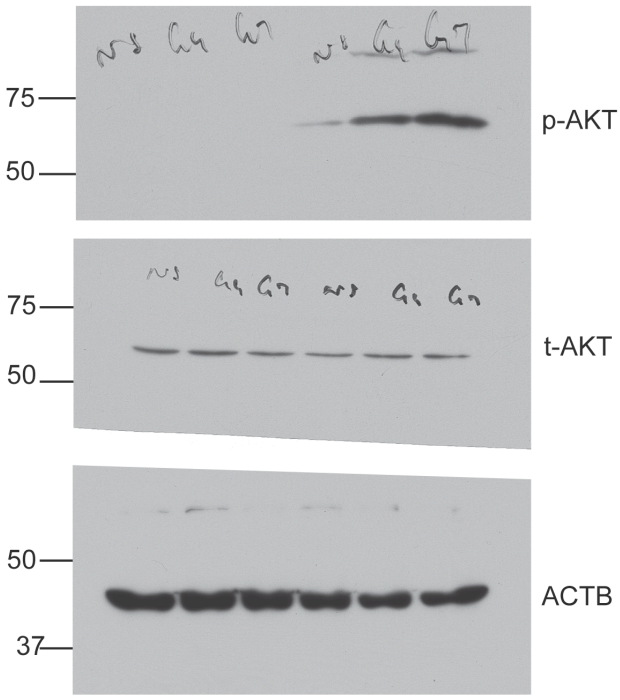


Figure 5a

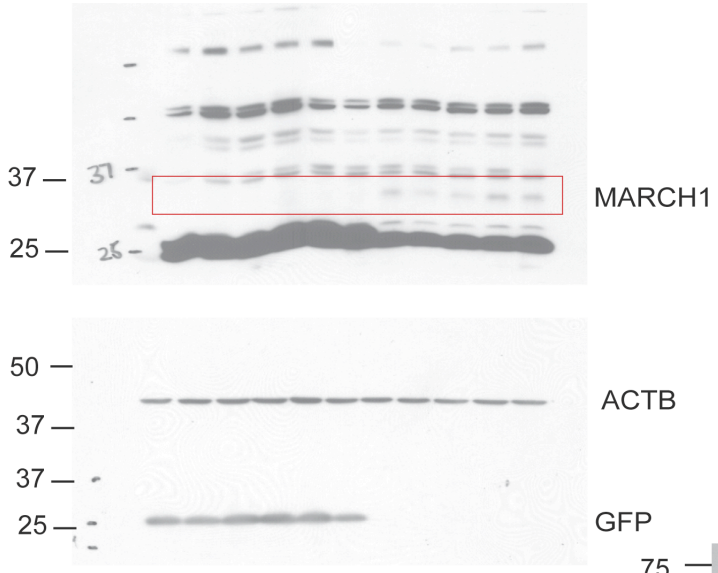


Figure 7a

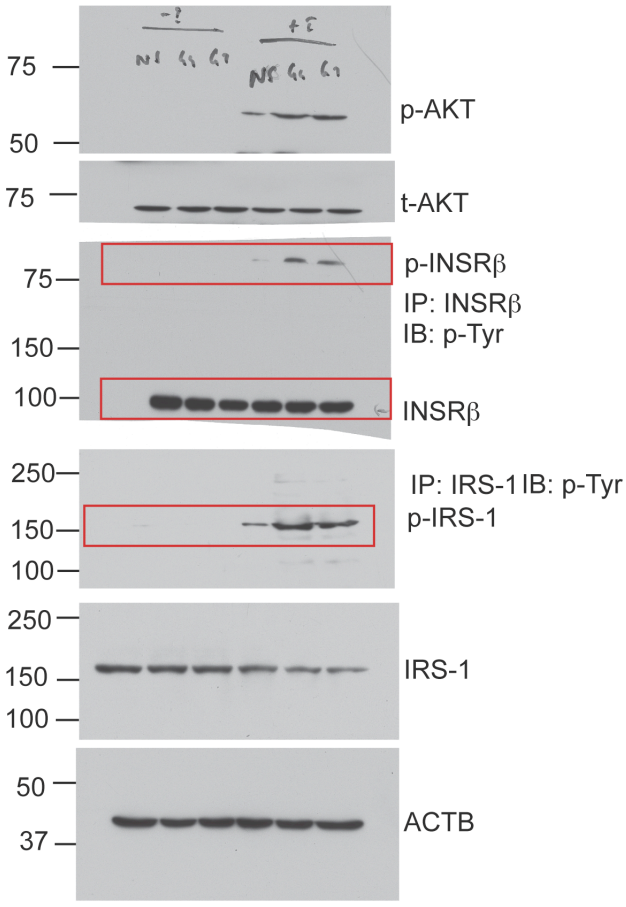


Figure 7b

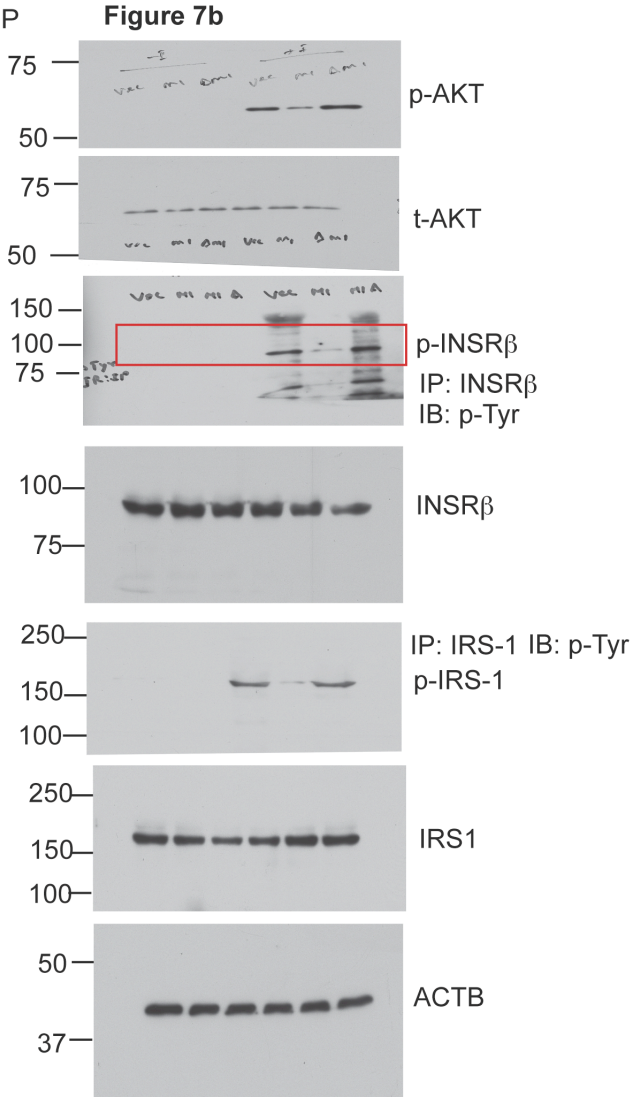
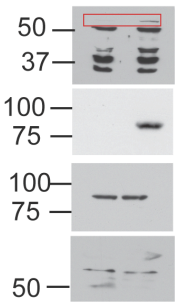


Figure 8a



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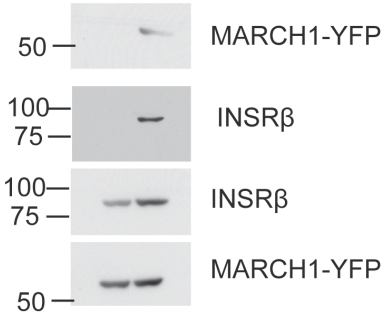

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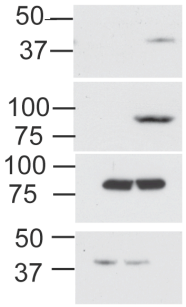

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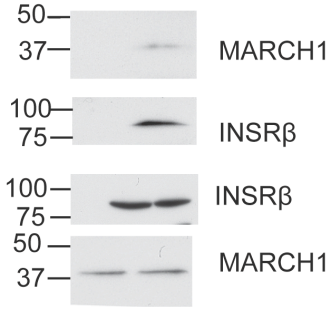

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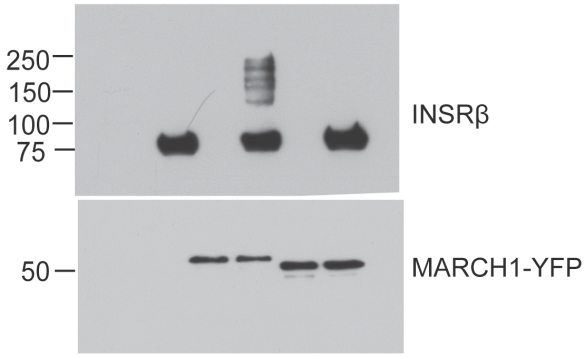

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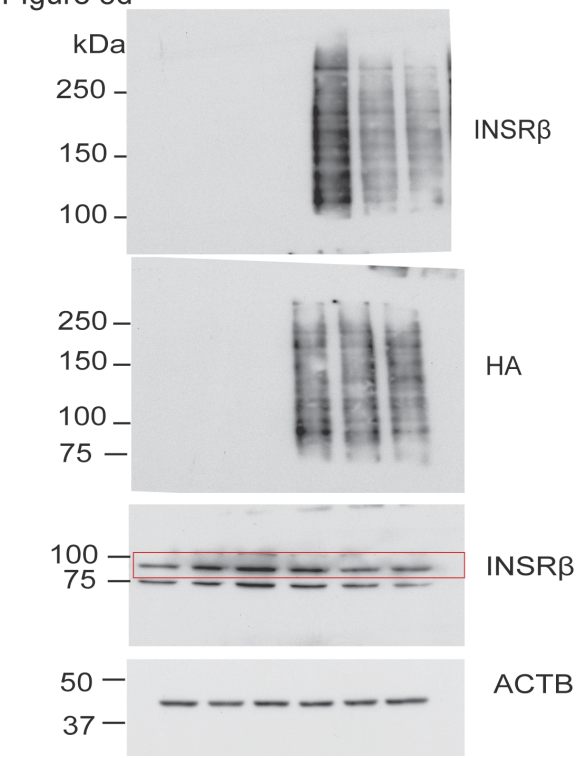

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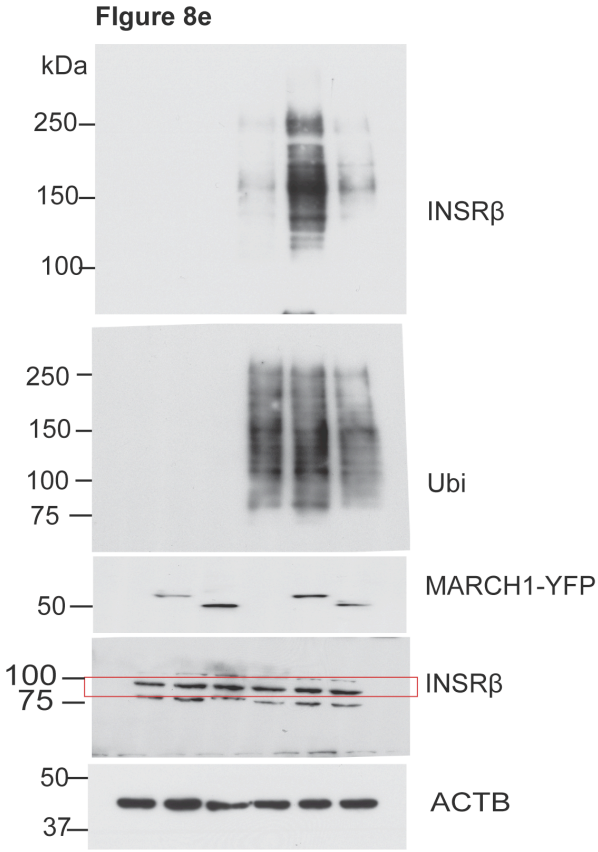

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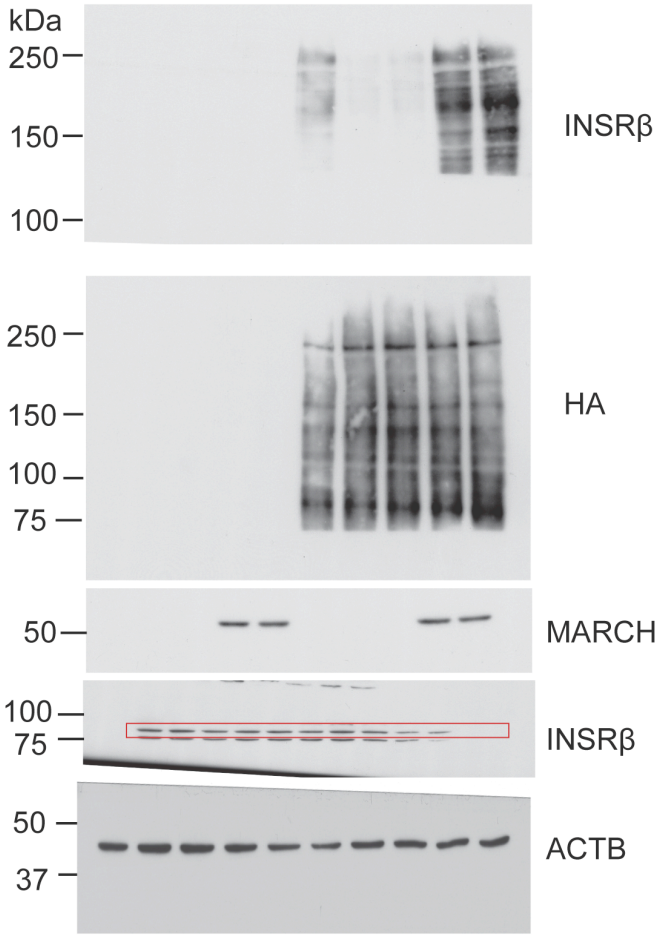
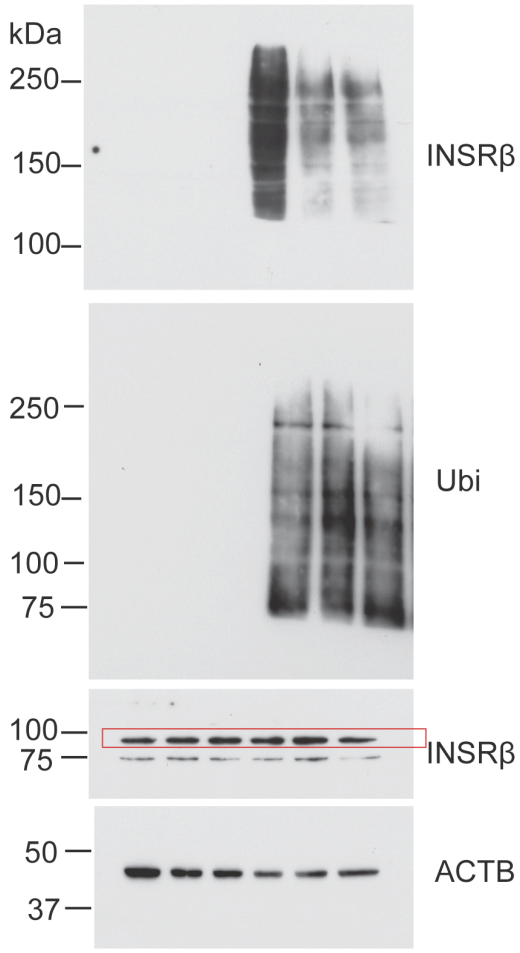


Figure 8g



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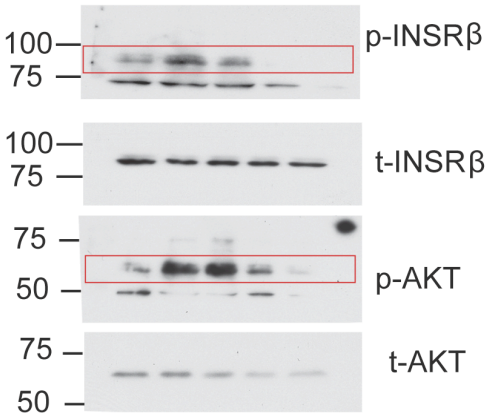
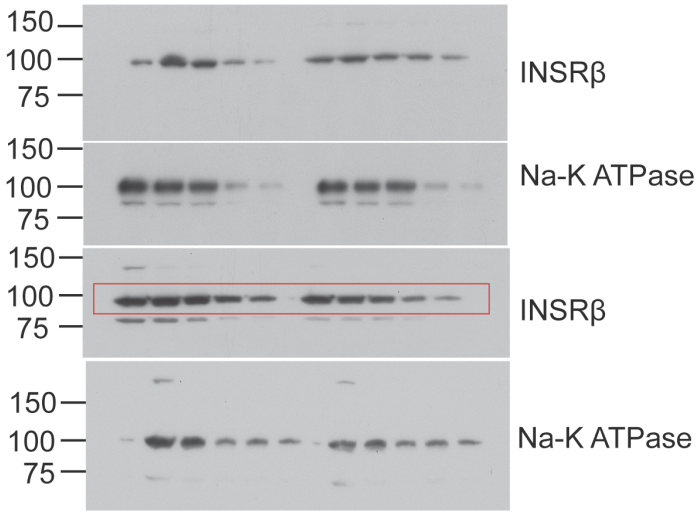


Figure 9a



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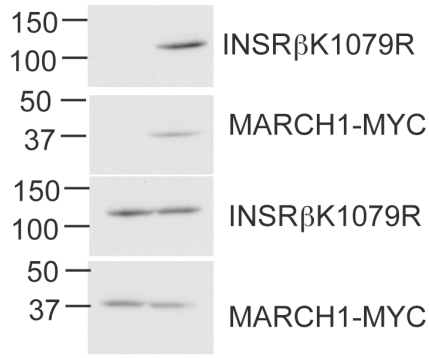

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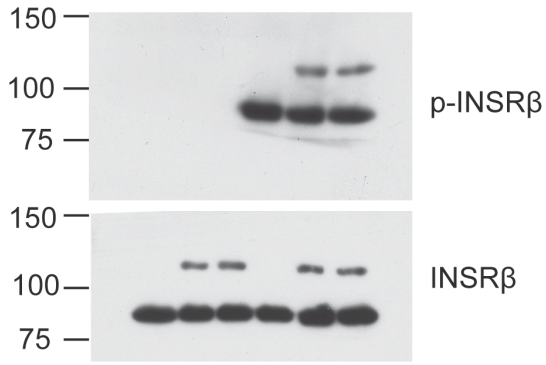

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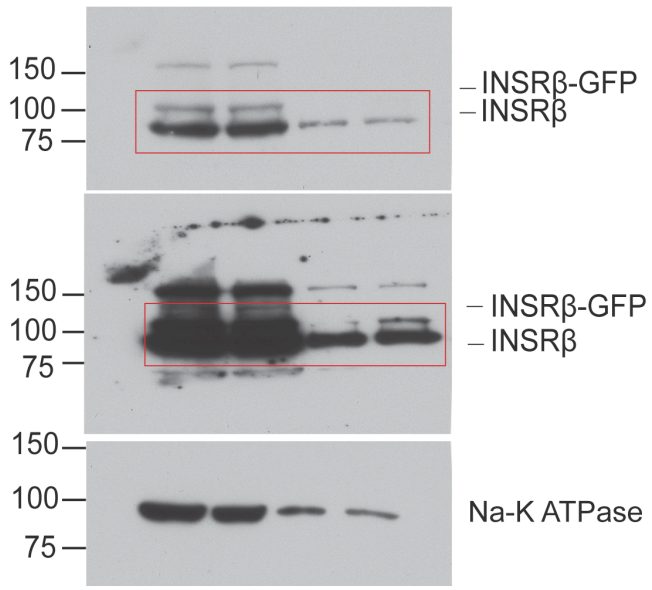
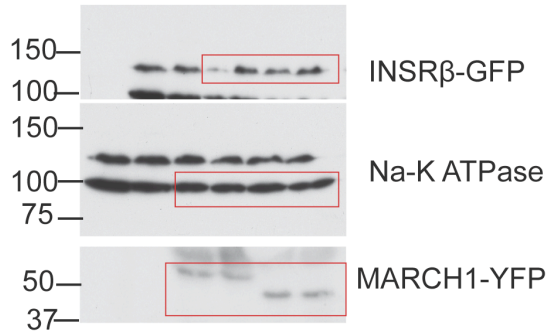
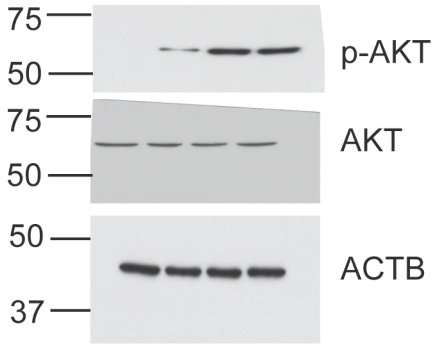


Figure 9g



Suppl. Fig. 1b



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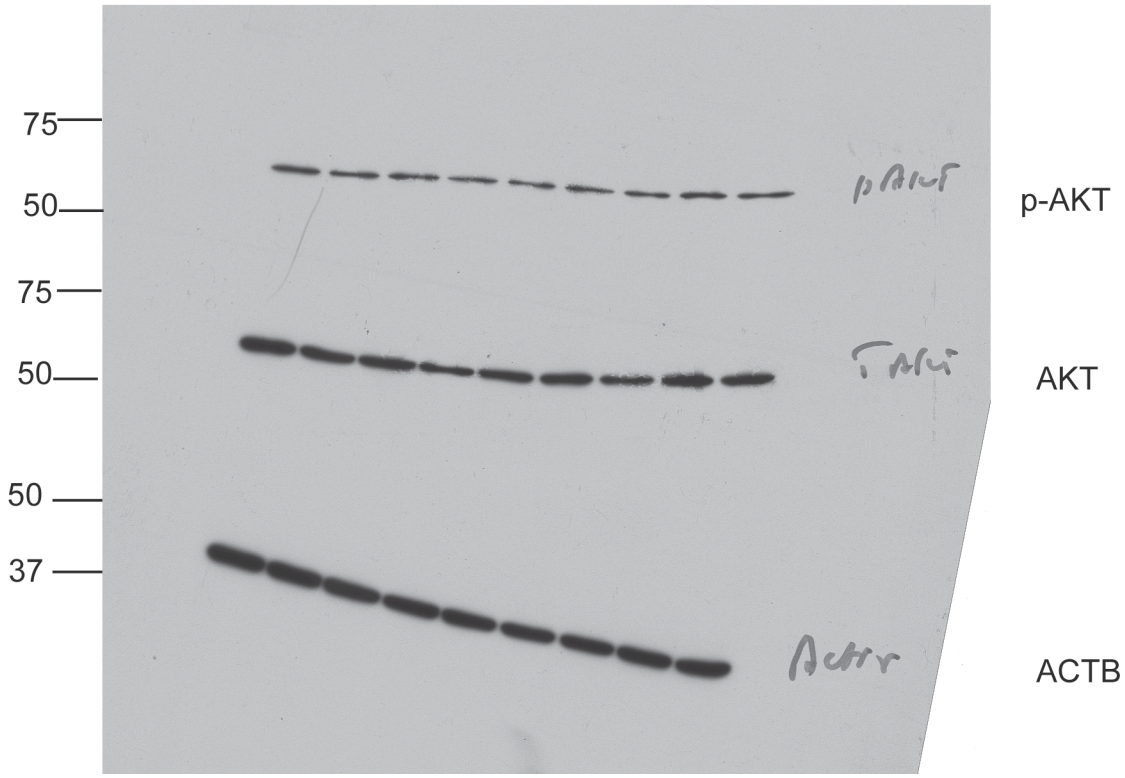

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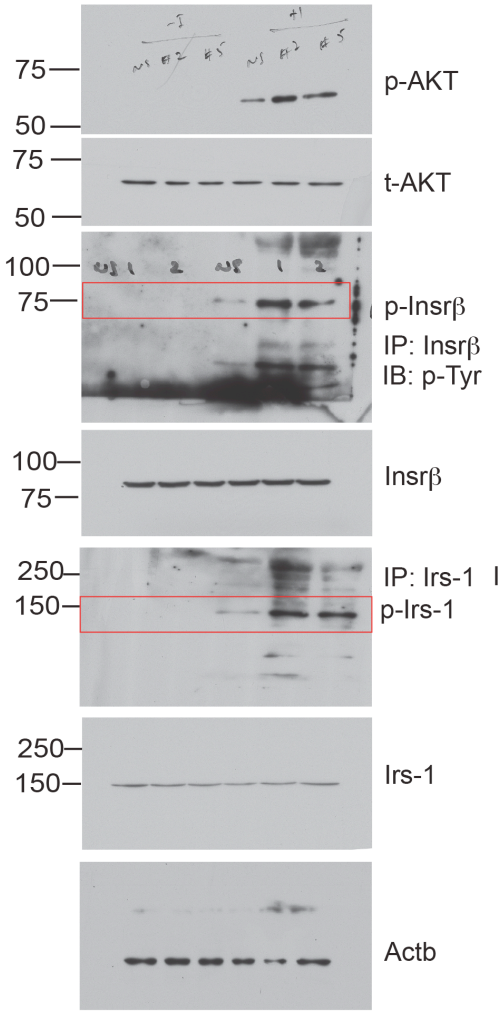

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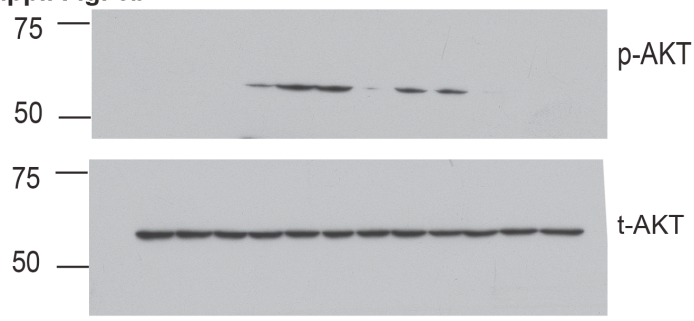

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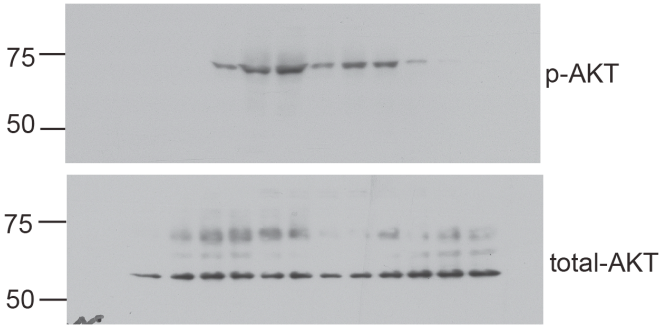
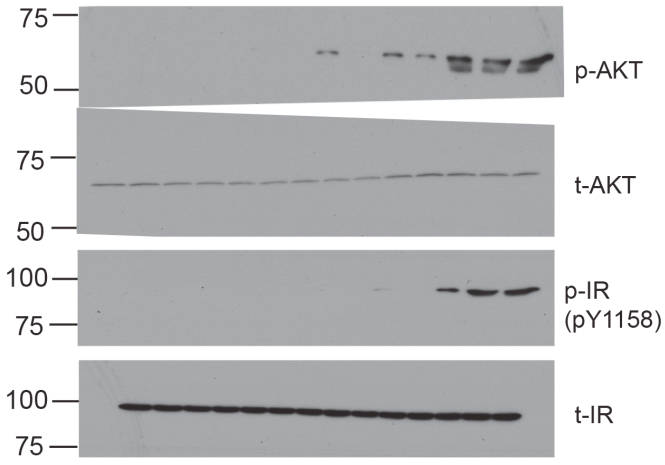
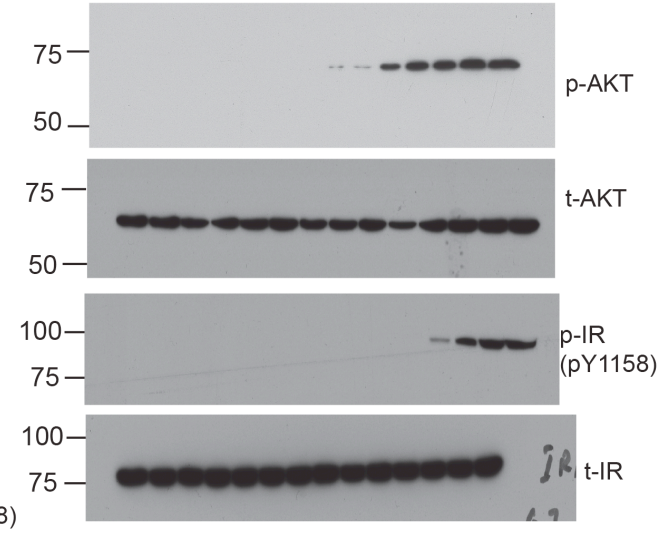


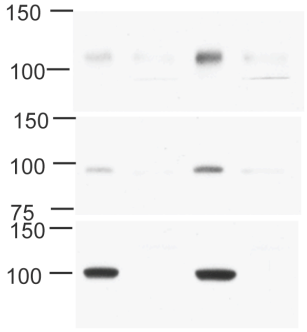
Suppl. Fig. 6d



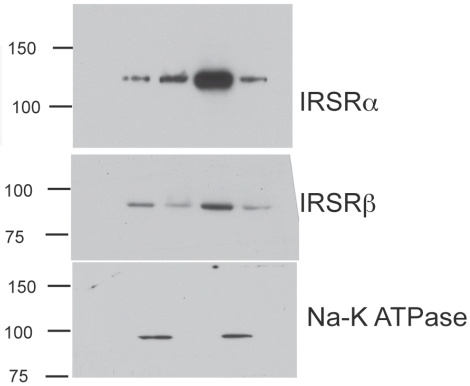
Suppl. Fig. 6e



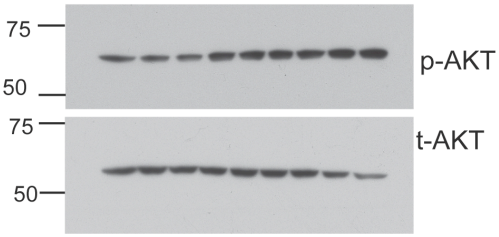
Suppl. Fig. 7a



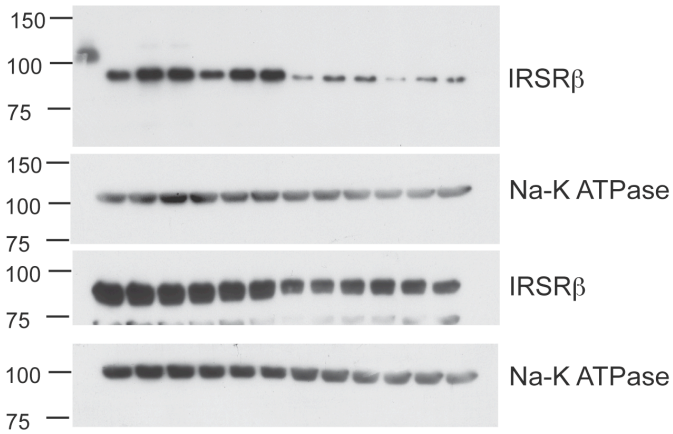
Suppl. Fig. 7b



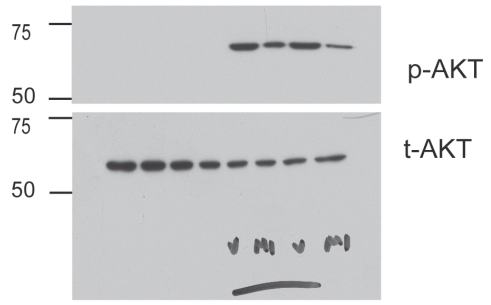
Suppl. Fig. 7c



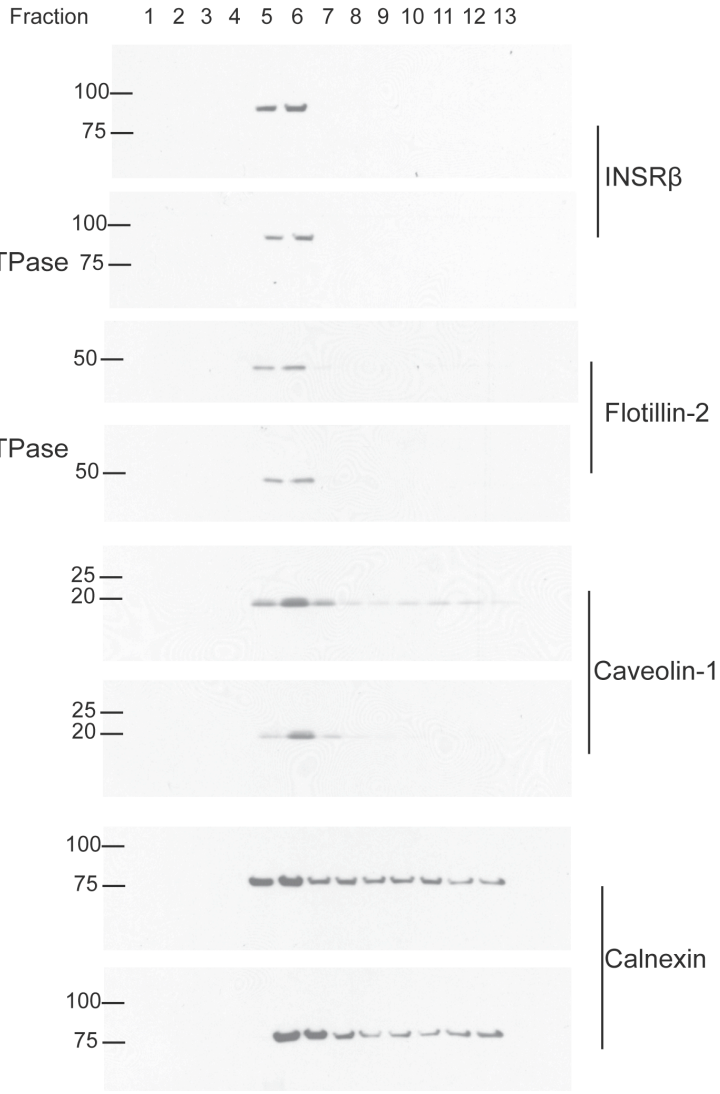
Suppl. Fig. 7d



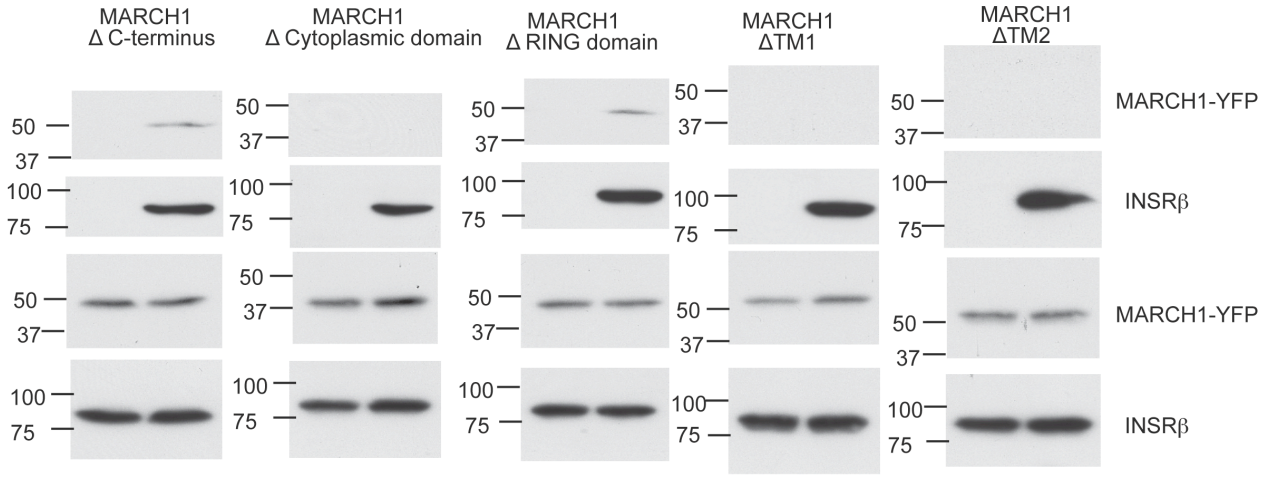
Suppl. Fig. 7e



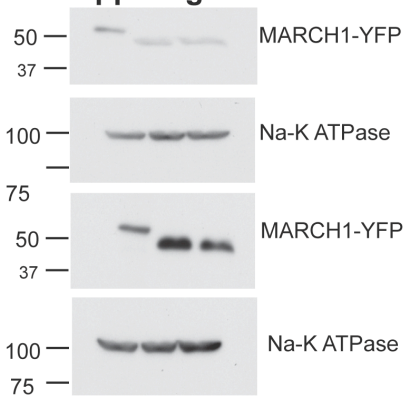
Suppl. Fig. 7f



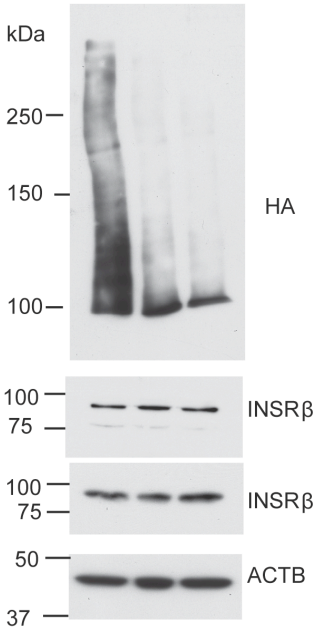
Suppl. Fig. 8b



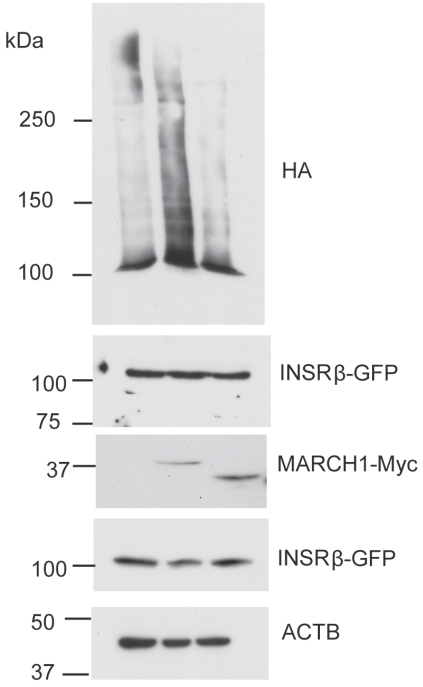
Suppl. Fig. 8c



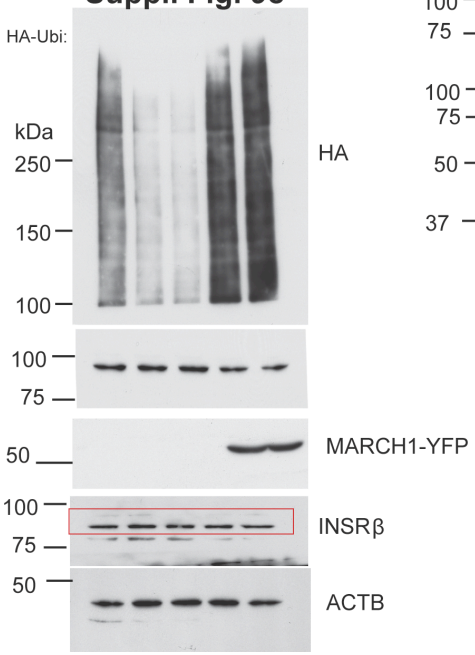
Suppl. Fig. 9a



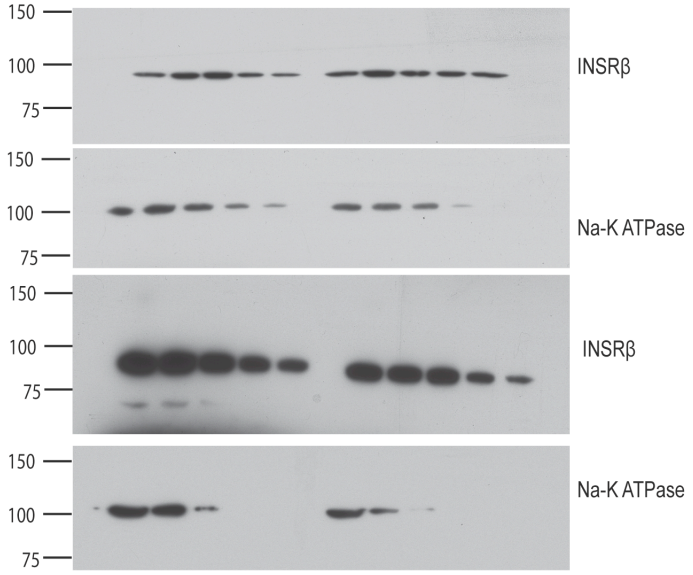
Suppl. Fig. 9b



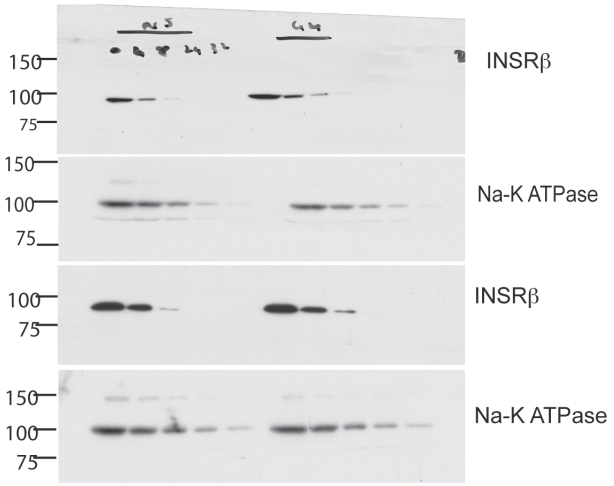
Suppl. Fig. 9c



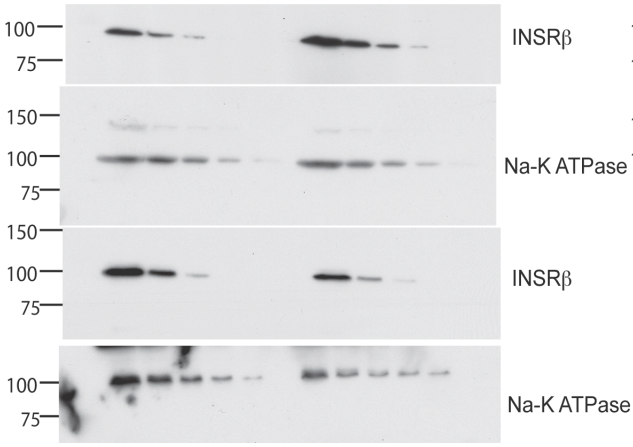
Suppl. Fig. 10a



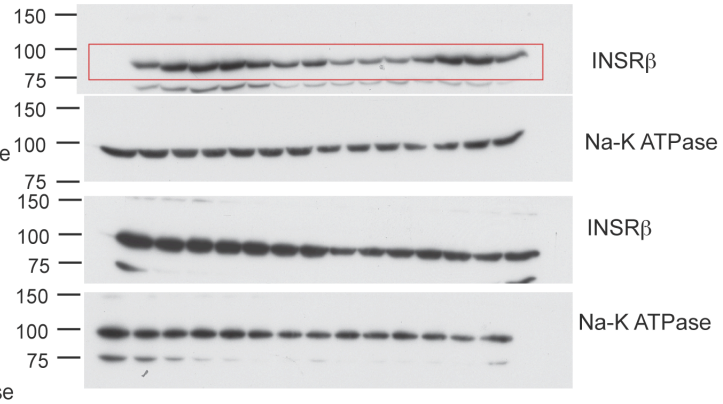
Suppl. Fig. 10c



Suppl. Fig. 10e



Suppl. Fig. 10g



Supplementary Figure 12. Uncropped images of western blot analysis.

Supplementary Table 1. List of E3 ligase/adapter genes targeted by the shRNAs in the large-scale RNAi screen

Gene Name	Accession Number
ANKIB1	NM_019004
ANAPC11	NM_001002244 NM_001002245 NM_001002246 NM_001002247 NM_001002248 NM_001002249 NM_016476
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ARIH2	NM_006321
AMFR	NM_001144
'MARCH7	NM_022826
BIRC2	NM_001166
BIRC3	NM_001165 NM_182962
BIRC4	NM_001167
BIRC7	NM_022161 NM_139317
BIRC8	NM_033341
BFAR	NM_016561
BMI1	NM_005180
BRAP	NM_006768
BARD1	NM_000465
BRCA1	NM_007294 NM_007295 NM_007296 NM_007297 NM_007298 NM_007299 NM_007300 NM_007302 NM_007303 NM_007304 NM_007305
CBL	NM_005188
CBLB	NM_170662
CBLC	NM_012116
CBLL1	NM_024814
CNOT4	NM_001008225 NM_013316
CGRRF1	NM_006568
CHFR	NM_018223
C1orf164	NM_018150
C13orf7	NM_024546
C14orf4	NM_024496
C16orf28	NM_023076
KIAA1542	NM_020901
CCNB1IP1	NM_021178 NM_182849 NM_182851 NM_182852
DPF1	NM_004647
DCST1	NM_152494
DTX3	NM_178502
DTX3L	NM_138287
DTX4	NM_015177
DTX1	NM_004416
DTX2	NM_020892
FANCL	NM_018062
GTF2H2	NM_001515
HLTF	NM_003071 NM_139048
IBRDC1	NM_152553
IBRDC2	NM_182757

IBRDC3	NM_153341
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LNX1	NM_032622
LNX2	NM_153371
LINCR	NM_001080535
LONRF1	NM_152271
LONRF2	NM_198461
LONRF3	NM_001031855 NM_024778
MGRN1	NM_015246
MKRN1	NM_013446
MKRN2	NM_014160
MKRN3	NM_005664
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MDM4	NM_002393
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MIB2	NM_080875
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MYCBP2	NM_015057
MYLIP	NM_013262
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NHLRC1	NM_198586
NSMCE1	NM_145080
NFX1	NM_002504 NM_147133 NM_147134
NFXL1	NM_152995
OSTM1	NM_014028
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PARK2	NM_004562 NM_013987 NM_013988
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PXMP3	NM_000318 NM_001079867
PEX10	NM_002617 NM_153818
PHF7	NM_016483 NM_173341

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RNF141	NM_016422
RNF144	NM_014746
RNF145	NM_144726
RNF146	NM_030963
RNF148	NM_198085
RNF149	NM_173647
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TRAIP	NM_005879
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UNK	NM_001080419
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FBXL6	NM_012162 NM_024555
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SOCS2	NM_003877
SOCS3	NM_003955
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SOCS6	NM_004232
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WSB2	NM_018639
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ABTB2	NM_145804
ANKFY1	NM_016376 NM_020740
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BTBD1	NM_001011885 NM_025238
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BTBD7	NM_001002860 NM_018167
BTBD8	NM_183242
BTBD9	NM_152733
BACH1	NM_001011545 NM_001186 NM_206866
BACH2	NM_021813
CCIN	NM_005893
C16orf44	NM_024731
ENC1	NM_003633
LOC388419	NM_001080466
GZF1	NM_022482
GMCL1	NM_178439
GAN	NM_022041
HIC1	NM_001098202 NM_006497
HIC2	NM_015094
LOC339745	NM_001001664
C8ORFK36	NM_001081675
MGC23270	NM_152646
IVNS1ABP	NM_016389 NM_006469
IBTK	NM_015525
IPP	NM_005897
KCNRG	NM_173605 NM_199464
KCTD1	NM_198991
KCTD10	NM_031954
KCTD12	NM_138444
KCTD13	NM_178863
KCTD14	NM_023930
KCTD15	NM_024076
KCTD16	NM_020768
KCTD17	NM_024681
KCTD2	NM_015353
KCTD20	NM_173562
KCTD21	NM_001029859

KCTD3	NM_016121
KCTD4	NM_198404
KCTD5	NM_018992
KCTD6	NM_153331
KCTD7	NM_153033
KCTD8	NM_198353
KCTD9	NM_017634
KCNV1	NM_014379
KCNS1	NM_002251
KCNS2	NM_020697
KCNS3	NM_002252
KCNB1	NM_004975
KCNB2	NM_004770
KCNA1	NM_000217
KCNA10	NM_005549
KCNA2	NM_004974
KCNA3	NM_002232
KCNA4	NM_002233
KCNA5	NM_002234
KCNA6	NM_002235
KCNA7	NM_031886
KCND1	NM_004979
KCND2	NM_012281
KCND3	NM_004980 NM_172198
KCNC1	NM_004976
KCNC2	NM_139136 NM_139137 NM_153748
KCNC3	NM_004977
KCNC4	NM_001039574 NM_004978 NM_153763
KCNG1	NM_002237 NM_172318
KCNG3	NM_133329 NM_172344
KLHDC5	NM_020782
KLHDC6	NM_207335
KBTBD10	NM_006063
KBTBD11	NM_014867
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KBTBD3	NM_152433 NM_198439
KBTBD4	NM_016506 NM_018095
KBTBD5	NM_152393
KBTBD6	NM_152903
KBTBD7	NM_032138
KBTBD8	NM_032505
KLHL1	NM_020866
KLHL10	NM_152467
KLHL11	NM_018143
KLHL12	NM_021633
KLHL13	NM_033495

KLHL14	NM_020805
KLHL15	NM_030624
KLHL17	NM_198317
KLHL18	NM_025010
KLHL20	NM_014458
KLHL21	NM_014851
KLHL22	NM_032775
KLHL23	NM_144711
KLHL24	NM_017644
KLHL25	NM_022480
KLHL26	NM_018316
KLHL28	NM_017658
KLHL29	XM_001134449 XM_940375
KLHL3	NM_017415
KLHL30	NM_198582
KLHL31	NM_001003760
KLHL32	NM_052904
KLHL34	NM_153270
KLHL4	NM_019117 NM_057162
KLHL5	NM_001007075 NM_015990 NM_199039
KLHL6	NM_130446
KLHL7	NM_001031710 NM_018846
KLHL8	NM_020803
KLHL9	NM_018847
KEAP1	NM_012289 NM_203500
LGALS3BP	NM_005567
LZTR1	NM_006767
MYNN	NM_018657
PATZ1	NM_014323 NM_032050 NM_032051 NM_032052
RCBTB1	NM_018191
RCBTB2	NM_001268
RHOBTB1	NM_001032380 NM_014836 NM_198225
RHOBTB2	NM_015178
RHOBTB3	NM_014899
SHKBP1	NM_138392
LOC123103	XM_063481
SPOP	NM_001007226 NM_001007227 NM_001007228 NM_001007229 NM_001007230 NM_00356
TNFAIP1	NM_021137
ZBTB1	NM_014950
ZBTB10	NM_023929
ZBTB11	NM_014415
ZBTB12	NM_181842
ZBTB16	NM_001018011 NM_006006
ZBTB17	NM_003443
ZBTB2	NM_020861
ZBTB20	NM_015642

ZBTB22	NM_005453
ZBTB24	NM_014797
ZBTB25	NM_006977
ZBTB26	NM_020924
ZBTB3	NM_024784
ZBTB32	NM_014383
ZBTB33	NM_006777
ZBTB37	NM_032522
ZBTB38	NM_001080412
ZBTB39	NM_014830
ZBTB4	NM_020899
ZBTB40	NM_001083621 NM_014870
ZBTB41	NM_194314
ZBTB43	NM_014007
ZBTB44	NM_014155
ZBTB45	NM_032792
ZBTB46	NM_025224
ZBTB48	NM_005341
ZBTB5	NM_014872
ZBTB6	NM_006626
ZBTB7A	NM_015898
ZBTB7B	NM_015872
ZBTB7C	NM_001039360
ZBTB8	NM_001040441
ZBTB9	NM_152735
ZNF131	NM_003432
ZFP161	NM_003409
ZNF238	NM_006352 NM_205768
ZNF295	NM_001098402 NM_001098403 NM_020727
ZNF509	NM_145291
FLJ30092	XM_497354 XM_942665
HACE1	NM_020771
HERC1	NM_003922
HERC2	NM_004667
HERC3	NM_014606
HERC4	NM_001017972 NM_015601 NM_022079
HERC5	NM_016323
HERC6	NM_001013000 NM_001013002 NM_001013005 NM_017912
HECTD1	NM_015382
HECTD2	NM_173497 NM_182765
HECTD3	NM_024602
HECW1	NM_015052
HECW2	NM_020760
HUWE1	NM_031407
UBE3A	NM_000462 NM_130838 NM_130839
ITCH	NM_031483

KIAA0317	NM_001039479
KIAA1333	NM_017769
NEDD4	NM_006154 NM_198400
NEDD4L	NM_015277
SMURF1	NM_020429 NM_181349
SMURF2	NM_022739
TRIP12	NM_004238
UBR5	NM_015902
UBE3B	NM_130466 NM_183415
UBE3C	NM_014671
WWP1	NM_007013
WWP2	NM_007014 NM_199423 NM_199424
CPSF1	NM_013291
DDB1	NM_001923
SF3B3	NM_012426
ANUBL1	NM_174890
OTUD7A	NM_130901
OTUD7B	NM_020205
KCTD7	NM_153033
RABGEF1	NM_014504
TNFAIP3	NM_006290
ZFAND3	NM_021943
ZFAND5	NM_006007
ZFAND6	NM_019006

Supplementary Table 2: List of genes for which multiple shRNAs were isolated from the RNAi screen.

Gene Symbol	Gene Name	Protein family
MARCH1	Membrane-associated ring finger (C3HC4) 1	MARCH
MARCH9	Membrane-associated ring finger (C3HC4) 9	MARCH
NHLRC1	NHL repeat containing E3 ubiquitin protein ligase 1	
KCNB1	Potassium voltage-gated channel, Shab-related subfamily, member 1	Potassium voltage-gated channel, Shab-related
RNFT1	Ring finger protein, transmembrane 1	
RNF166	Ring finger protein 166	
DTX3L	Deltex 3-like (Drosophila)	DELTEX

Supplementary Table 3. Primers were used for qRT-PCR analysis, ChIP experiments, and cloning. The antibodies were used as indicated. The source and concentrations of chemical inhibitors and biologics used for drug treatment experiments are summarized.

Application	Gene symbol	Species	Forward Primer (5'-3')	Reverse Primer (5'-3')
qPCR	MARCH1	Human	GCCTCACAAACCTCCACATT	TCTGCAGATGTCCTGAGTGG
	MARCH9	Human	TGGAGCTGTGAGCTCTGCTA	CTGAAGGGCTGAGTGAGGAC
	NHLRC1	Human	GTCACCATCACCAACGACTG	TCTCCACACCCCAAGGTAAG
	KCNB1	Human	TGCCAGTGGTGGTAGGTTTG	GAGGAGTGGACTGGGGTCAC
	RNF166	Human	GAAGGTCCAGGAGCAGATGG	TGGCTTTCCACACAGTGCTT
	RNFT1	Human	ATGCCTCAACCCCTCAGTGT	GAGTGACCCCGTAAGCGACT
	DTX3L	Human	TGTCCCACATGCCAGACTTC	TTTCCTGGGTTTGGGTGTTC
	ACTIN	Human	GCATGGAGTCCTGTGGCATC	TTCTGCATCCTGTCGGCAAT
	MARCH1	Mouse	CAGATGACCACGAGCGAAAG	AAGACGAGACCTCCCGTGAA
	MARCH9	Mouse	GTGACGTTCCAGTTCCCACA	TCTGGCATTGGTCTCCCTTT
	NHLRC1	Mouse	GTCACCAACGACTGCCATGT	GCCTCTGCATCAGTCACCAG
	KCNB1	Mouse	GATGGCCAAGACCCAGTCTC	GCTGATGAAGCTGTCGATGC
	RNF166	Mouse	GTGAAGCACTGCGTGGAAAG	CTGGAAGGCAGCTTCTTCGT
	RNFT1	Mouse	CCCACAGTGAAGCAAGACCA	AGCAGCCCAATTCCAAGAGA
	DTX3L	Mouse	GCATCCAGAAAGGGAACCAG	TCGCCGTGTTCCATGATAAG
	ACTIN	Mouse	AGGCTCTTTTCCAGCCTTCC	GTCAGCAATGCCTGGGTACA
	MARCH1	Rat	ACAGGAACCTTGCGCTTTGT	CTTTCGCTCGTGGTCATCTG
	FOXO1	Human	GAGCAGCTGCAATGGCTATG	TGTGTGGGAAGCTTTGGTTG
	CBL	Human	GAGGCCATGGCTCTGAAATC	AGCCGAGCTTTCACTTCGTC
	NEDD4	Human	CCACCAGGTTGGGAAGAAAA	ACCTGCTGGCCTGAATCACT
ChIP				

	MARCH1	Human	TTGAGGTCCCCTC GTTGTTT	TGGCTGCTCAGTAGCTC AGG
Cloning				
Luciferase reporter	MARCH1 promoter	Human	ATGCGGTACCGCT GAATATTACTCCA TTGT	ATGCGTCGACTGACATC TCTCGCCCAGGAG
AAV	MARCH1	Mouse	AGCGGATCCACCA TGGATTACAAGGA TGACGATGACAAG ATGCTGGGCTGGT GTGAAGCG	TCAGACTGATACAACTT CAGG
Mutagenesis				
	MARCH1 promoter		CTCAGACTCCCGC CTGTACCAGTACA CATCTTTCTCCCT	AGGGAGAAAGATGTGTA CTGGGTACAGGCGGGAG TCTGAG
	INSR K1079R		AATGAGGCCTCGG TCATGAGGGGCTT CACCTGCCATCAC GTG	CACGTGATGGCAGGTGA AGCCCCTCATGACCGAG GCCTCATT
	MARCH1 Transmembrane I Deletion		ATAATGGAGACCA AGCTCAAATTCAT AATGGAGACCAA GCTC	GAGCTTGGTCTCCATTAT GAATTTGAGCTTGGTCTC CATTAT
	MARCH1 Transmembrane II Deletion		TTGTATGTATTGA TAGACCGGGTGGT AGCCATTGGCTTC ACA	TGTGAAGCCAATGGCTA CCACCCGGTCTATCAAT ACATACAA
	MARCH1 shRNA#1 resistant cDNA		AATATTCTGTTCC GTTACCTTTCATG TAATCGCGATCAC CT	AGGTGATCGCGATTACA TGAAAGGTAACGGAACA GAATATT
	MARCH1 shRNA#2 resistant cDNA		GGAAAAAAAGTA AAATCTCCACTAT GTACTACCTTAAC CAAGA	TCTTGGTTAAGGTAGTA CATAGTGGAGATTTTAC TTTTTTTCC
	Protein symbol	**Species**	**Antibody, Cat. #**	**Application**
	AKT	Human, Mouse	Cell Signaling #4685	IB
	AKT pS473	Human, Mouse	Cell Signaling #4060	IB
	INSRα	Human, Mouse	Santa Cruz #sc-710	IB
	INSRα	Human, mouse	Pierce #83-14	Flow Cytometry
	INSRβ	Human, Mouse	Cell Signaling #3025	IP, IB

	INSRβ	Human, Mouse	Cell Signaling #3020	IP, IB
	INSRβ	Human, Mouse	Santa Cruz #sc-711	IP, IB
	IRS1	Human, Mouse	Santa Cruz #sc-559	IP, IB
	β-Actin	Human, Mouse	Cell Signaling #3700	IB
	INSR pY1158	Human, Mouse	Cell Signaling #3021	IB
	Na-K ATPase	Human, Mouse	Abcam #ab7671	IB
	pTyr	Human, Mouse	Cell Signaling #8954	IB
	MARCH1	Mouse	Sigma #3D2	IB
	MYC epitope	n/a	Santa Cruz #sc-40	IB
	GFP	n/a	Cell Signaling #2956	IB
	GFP	n/a	Cell Signaling #2955	IB
	GFP	n/a	Santa Cruz #sc-8334	IP, IB
	Flotillin-2	Human	Cell Signaling #3436	IB
	Caveolin-1	Human	Cell Signaling #3267	IB
	Calnexin	Human	Abcam #22595	IB
	FoxO1	Human	Cell Signaling #2880	ChIP
	Ubiquitin	Multiple species	Biomol #PW8810	IP, IB
	HA-Tag	n/a	Cell signaling #3724	IP, IB
	Inhibitor		**Source**	
	Cycloheximide	100µg/ml	Sigma #C1988	
	Cytochalasin B	5 µM	Sigma #C6762	
	PTP1B inhibitor	10µM	Calbiochem #539749	
	Dynasore	20µM	Calbiochem # 324410	
	Chemicals /Biologics			
	Apo Transferrin	5 µg/ml	Sigma #T1147	
	Dexamethasone	0.25 µM	Sigma #D4902	
	EGF	50 ng/ml	Sigma #E9644	
	FGF	10 ng/ml	Sigma #SRP3043	
	Fibronectin	0.5µg/ml	Sigma, #F1141	
	Hydrocortisone	100nM	Sigma #H0888	
	Insulin	0.05µg/ml	Sigma #D4902	

	Isobutylmethylxanthine	0.5 mM	Sigma #I7018	
	N-ethyl maleimide	2mM	Sigma #3876	
	Selenium	5ng/ml	Sigma #S5261,	
	USP2	1μM	Boston Biochem #E506	

Supplementary Table 4. shRNA IDs for the shRNAs used in this study

Gene Name	Species	shRNA IDs	
		shRNA#1	**shRNA#2**
MARCH1	Human	TRCN0000037019	TRCN0000037022
MARCH9	Human	TRCN0000073170	TRCN0000073171
NHLRC1	Human	TRCN0000034184	TRCN0000034186
KCNB1	Human	TRCN0000044783	TRCN0000044785
RNF166	Human	TRCN0000007801	TRCN0000007803
RNFT1	Human	TRCN0000034332	TRCN0000034333
DTX3L	Human	TRCN0000073208	TRCN0000073210
FOXO1	Human	TRCN0000039578	TRCN0000010333
CBL	Human	TRCN0000039723	TRCN0000039724
NEDD4	Human	TRCN0000007553	TRCN0000007554
March1	Mouse	V3LMM_461750	TRCN0000176313