## Appendix Figures

### Appendix Materials and Methods

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Appendix Figure S1. Confirmation of knockdown efficiencies and expression of ADIPOR2 constructs. (A) Cells (gCTR or gRNF145) in used for experiments in Figure 5A were analysed by immunoblotting before treatment with FAs. LE, long exposure; *non-specific bands. (B) Immunoblot analysis of cells used in Figure 5C. ADIPOR2-depleted cells were stably complemented with epitope-tagged WT ADIPOR2 (ADIPOR2-HA) or a catalytically inactive ADIPOR2 mutant (dead ADIPOR2-HA). Expression levels of recombinant ADIPOR2 variants before exposure to FAs were determined by immunoblot analysis using a specific anti-HA antibody.
Appendix Figure S2. RNF145/ADIPOR2 knockdown efficiencies in cells used for FRAP and SMT analysis. (A) CRISPR/Cas9-mediated stable depletion of RNF145 (gRNF145), ADIPOR2 (gADIPOR2), or B2M (gCTR) in HEK-293T cells used in FRAP analysis (Figure 6A). (B) CRISPR/Cas9-mediated stable depletion of RNF145 (gRNF145), ADIPOR2 (gADIPOR2), or B2M (gCTR) in U2-OS cells used for SMT analysis (Figure 6B). Cell lines were generated by transfecting U2-OS cells with pools of 4 sgRNAs against the indicated targets. Knockdown cell pools were selected by puromycin for 72h.
Appendix Material & Methods

(A) MS Method - TMT

| Start Time (min): | 0 |
| End Time (min): | 190 |
| Cycle Time (sec): | 3 |

**Master Scan**
- MS/MS
  - Detector Type: Orbitrap
  - Orbitrap Resolution: 120 000
  - Mass Range: Normal
  - Use Quadrupole Isolation: True
  - Scan Range (m/z): 400-1000
  - RF Level (%): 60
  - AGC Target: Custom
  - Normalized AGC Target (%): 125
  - Maximum Inject Time Mode: Custom
  - Maximum Inject Time (ms): 50

**Data Type:** Profile
- Polarities: Positive
- Source Fragmentation: Disabled
- Scan Description:

**Fibers:**
- Monoisotopic Peak Determination: Peptide
- Charge State: Include charge states: 2-7
- Include undetermined charge states: False
- Dynamic Exclusion: Use Common Exclusion Settings: False
- Exclude ion (m/z): 1
- Exclusion duration (s): 50
- Mass Tolerance (ppm): Low 10, High 10
- Excludes isotopes: True
- Isotope dependent centric on single charge state per precursor only: True

**Intensity**
- Filter Type: Intensity Threshold
- Intensity Threshold: 5000

**Data Dependent**
- Data Dependent Mode: Decoy Scan
- Cycle Time: 3
- Time between Master Scan (sec): 3

**Scan Event Type:** Scan
- **EASY-IC**
  - Isolation Mode: Quadrupole
  - Isolation Window (m/z): 0.7
  - Isolation Offset: Off

**Activation Type:** CID
- Collision Energy Mode: Fixed
- CD Collision Energy (eV): 35
- CD Activation Time (ms): 10
- Activation Q: 0.25
- Multistep Activation: False
- Detector Type: Ion Trap
- Ion Trap Scan Rate: Rapid
- Mass Range: Normal
- Scan Range (m/z): Auto
- AGC Target: Custom
- Normalized AGC Target (%): 80
- Maximum Inject Time Mode: Auto
- Maximum Inject Time (ms): 50

**Data Type:** Profile
- Use ISOF-IC**: False
- Scan Description:

**Filters:**
- **Precursor Selection Range**
  - Selection Range Mode: Mass Range
  - Mass Range (m/z): 400-2000
- **Precursor Ion Exclusion**
  - Exclusion Mass Width: 10
  - High: 7
- **Isotope Tag Loss Exclusion**
  - Related: TMT

**Data Dependent**
- Data Dependent Mode: Scan Per Outcome

**Scan Event Type:** Scan
- **EASY-IC**
  - MS Level: 3
  - Synchronous Precursor Selection: True
  - Number of ISPs: 10
  - MS Isolation Window (m/z): 2
  - MS2 Isolation Window (m/z): 2
  - Isolation Offset: Off
  - Activation Type: HCD
  - HCD Collision Energy (%): 65
  - Detector Type: Orbitrap
  - Orbitrap Resolution: 50 000
  - Mass Range: Normal
  - Scan Range Mode: Define m/z range
  - Scan Range (m/z): 100-1000
  - AGC Target: Custom
  - Normalized AGC Target (%): 40
  - Maximum Inject Time Mode: Custom
  - Maximum Inject Time (ms): 120
  - Microscan(s): 1
  - Data Type: Profile
  - Use ISOF-IC**: False
  - Scan Description:

**Number of Dependent Scans:** 3
# MS Method – AP-MS

| Parameter                  | Setting |
|----------------------------|---------|
| Start Time (min)           | 0       |
| End Time (min)             | 87      |
| Cycle Time (sec)           | 2       |

**Master Scan:**

- **MS OT**
  - Detector Type: Orbitrap
  - Orbitrap Resolution: 120000
  - Mass Range: Normal
  - Use Quadrupole Isolation: True
  - Scan Range (m/z): 400-1600
  - RF Lens (%): 60
  - AGC Target: Standard
  - Maximum Injection Time Mode: Custom
  - Maximum Injection Time (ms): 50
  - Microscans: 1
  - Data Type: Profile
  - Polarity: Positive
  - Source Fragmentation: Disabled
  - Scan Description:

**Filters:**

- **Intensity**
  - Filter Type: Intensity Threshold
  - Intensity Threshold: 5.0e3

- **MIPS**
  - Monoisotopic Peak Determination: Peptide

- **Dynamic Exclusion**
  - Use Common Settings: False
  - Exclude after n times: 1
  - Exclusion duration (s): 20
  - Mass Tolerance: ppm
  - Low: 5
  - High: 5
  - Exclude Isotopes: True
  - Perform dependent scan on single charge state per precursor only: True

- **Charge State**
  - Include charge state(s): 2-4
  - Include undetermined charge states: False

**Data Dependent**

- Data Dependent Mode: Cycle Time
  - Time between Master Scans (sec): 2

**Scan Event Type 1:**

- Scan:
  - didMS3 TT HCD
    - Isolation Mode: Quadrupole
    - Isolation Window (m/z): 1.6
    - Isolation Offset: Off
    - Activation Type: HCD
    - Collision Energy Mode: Fixed
    - HCD Collision Energy (%): 34
    - Detector Type: Ion Trap
    - Ion Trap Scan Rate: Rapid
    - Mass Range: Normal
    - Scan Range Mode: Auto
    - AGC Target: Standard
    - Maximum Injection Time Mode: Auto
    - Microscans: 1
    - Data Type: Centroid
    - Scan Description:
(B) Searching Schematic

1. Raw Files
2. Data Refinement
3. Search_1 (0.1% PSM FDR)
4. Search_2 (0.1% PSM FDR)
5. Search_3 (0.1% PSM FDR)
6. Quantitation

- Minimum one unique Peptide
- "Significant Peptides"

Data Refinement

- Merge Scans [DDA]
- Correct Precursor [DDA]
  - Mass only
  - Mass and Charge states
  - Min charges: 1, Max charges: 3
- Associate feature with chimera scan [DDA]
- Filter Features
  - Only keep features satisfying:
    - m/z between and
    - Retention time between and
    - Charge between 2 and 8

Search_1

- PEAKS Search
  - Error Tolerance: 10.0 ppm
  - Enzyme: Trypsin, N, K
  - Digest mode: Specific
  - Maximum missed cleavages per peptide: 3
  - PTMs: Carbamidomethylation, TMT, Dioxidation (M), Deamidation (N), Acetylation (Protein N-term)
  - Maximum allowed variable PTM per peptide: 3

- Database: Human_H_TMT16
- De Novo Tag Options: None
- General Options:
  - Estimate FDR with decay fusion
  - Find unspecified PTMs with PEAKS PTM
  - Find more mutations with SPOXER
Appendix Materials and Methods; Collection and processing of Mass spectrometry data. Workflow (A) and data processing (B) of data represented in Figure 1A & B and EV Fig 1A.