Supplementary Figure 1. Sequence alignment of E2 alpha-1 helix sequences that engage the UFD of E1. Alpha-1 helix residues are colored according to their hydrophobicity (yellow), hydrophilicity (green), and positive (blue) or negative (red) charge. Ellipses represent continuation of sequences.
| Data Set                  | E1       | E1:SAH-UBE2A | E1:SAH-UBE2A-R7E | E1:SAH-UBE2A-R7A/L9A/B10A |
|--------------------------|----------|--------------|-----------------|--------------------------|
| HDX reaction details     | Final D$_2$O concentration=94.7%, pH$_{read}$=7.60, 21 °C. See also footnote a |
| HDX time course          | 10s, 1m, 10m | 10 min     |
| HDX controls             | 7 undeuterated | 2 undeuterated | 1 undeuterated |
| Back-exchange            | 30-35% |
| Number of peptides       | 250 followed; 324 identified | 218 followed; 286 identified |
| Sequence coverage        | 82.5% |
| Average peptide length   | 12.5 a.a. | 12.0 a.a. |
| Redundancy               | Redundancy: 3.51 | Redundancy: 2.93 |
| Replicates               | 3 biological | 2 biological (1:25 E1:peptide) | 2 technical (1 each at 1:10 & 1:25, E1:peptide) | 2 technical (1 each at 1:10 & 1:25, E1:peptide) |
| Repeatability            | +/- 0.25 relative Da |
| Meaningful differences    | > 0.5 Da |

* a 18-fold dilution with labeling buffer [20 mM HEPES pD 8.0, 50 mM NaCl, 99.9% D$_2$O]. 1:1 dilution with quench buffer [0.8 M guanidinium chloride, 0.8% [v/v] formic acid, H$_2$O, pH 2.0]

Supplementary Table 1. Data summary and list of experimental parameters for HXMS analyses of UBE1 and the UBE1/SAH-UBE2A interactions.
| Peptide          | N-terminus | Sequence                                | C-terminus | MW  | (M+3)/3 | Figures |
|------------------|------------|-----------------------------------------|------------|-----|---------|---------|
| Ubc15-1          | Ac         | BPSSASRQLRKLQKLEIQ                      |            | 2136| 713.0   | 1, ED: 1 |
| SAH-Ubc-2        | Ac         | BPSSXSRQLRKLQKEIQ                       |            | 2202| 734.9   | 1       |
| SAH-Ubc15-3      | Ac         | BPSSAXRQLRKLQKEIQ                       |            | 2186| 729.6   | 1       |
| SAH-Ubc15-4      | Ac         | BPSSASRXLRLQKLEIQ                       |            | 2130| 710.9   | 1       |
| SAH-Ubc15-5      | Ac         | BPSSASRXLRLQKLEIQ                       |            | 2145| 715.9   | 1       |
| SAH-Ubc15-6      | Ac         | BPSSASRXLRLQKXKEIQ                      |            | 2160| 720.9   | 1       |
| SAH-Ubc15-7      | Ac         | BPSSASRXLRLQKXKEIQ                      |            | 2129| 710.6   | 1       |
| SAH-Ubc15-8      | Ac         | BPSSASRXLRLQKXKEQ                       |            | 2145| 715.9   | 1       |
| SAH-Ubc15-9      | Ac         | BPSSASRXLRLQKXKEIX                      |            | 2145| 715.9   | 1       |
| SAH-Ubc15-10     | Ac         | BPSS8SRQLRKLQKEIQ                       |            | 2229| 744.0   | 1       |
| SAH-Ubc15-11     | Ac         | BPSSAS8QLRKLQKEIQ                       |            | 2213| 738.6   | 1, 2, ED: 1 |
| SAH-Ubc15-12     | Ac         | BPSSAS8QLRKLQKEIQ                       |            | 2159| 720.6   | 1       |
| SAH-Ubc15-13     | Ac         | BPSSAS8QLRKLQKEIQ                       |            | 2172| 724.9   | 1       |
| SAH-Ubc15-14     | Ac         | BPSSAS8QLRKLQKEIQ                       |            | 2186| 729.6   | 1       |
| SAH-Ubc15-15     | Ac         | BPSSAS8QLRKLQKEIQ                       |            | 2202| 734.9   | 1       |
| SAH-UBE2D2       | Ac         | BSTPB8LRIHKXQLNLAR                     |            | 2038| 680.2   | 2       |
| SAH-UBE2G2       | Ac         | BAGTB8LRKLBAXYK                       |            | 1679| 560.8   | 2       |
| UBE2A            | Ac         | BSTPARRRRLBRXFKRLQ                     |            | 2140| 714.3   | 2, ED: 2 |
| SAH-UBE2A        | Ac         | BSTPB8RRRLBRXFKRLQ                     | Lys(FITC) | 2627| 876.7   | 2, ED: 2, 7 |
| SAH-UBE2A        | Ac         | BSTPARRRRLBRXFKRLQ                     | Lys(biotin)| 2790| 931.1   | 2       |
| SAH-UBE2A-B1A    | Ac         | BSTPB8RRRLBRXFKRLQ                     |            | 2231| 744.7   | 3       |
| SAH-UBE2A-S2A    | Ac         | BSTPB8RRRLBRXFKRLQ                     |            | 2257| 753.4   | 3       |
| SAH-UBE2A-T3A    | Ac         | BSAP8RRRLBFKRLQ                         |            | 2243| 748.7   | 3       |
| SAH-UBE2A-P4A    | Ac         | BSA8RRRLBFKRLQ                         |            | 2247| 750.0   | 3       |
| SAH-UBE2A-R6A    | Ac         | BSTP8ARRRLBFKRLQ                      |            | 2188| 730.3   | 3       |
| SAH-UBE2A-R7A    | Ac         | BSTP8ARRRLBFKRLQ                      |            | 2188| 730.3   | 3       |
| SAH-UBE2A-R8A    | Ac         | BSTP8RRRBFKRLQ                         |            | 2188| 730.3   | 3       |
| SAH-UBE2A-L9A    | Ac         | BSTP8RRRBFKRLQ                         |            | 2231| 744.7   | 3       |
| SAH-UBE2A-B10A   | Ac         | BSTP8RRRLBFKRLQ                        |            | 2231| 744.7   | 3       |
| SAH-UBE2A-R11A   | Ac         | BSTP8RRRLBFKRLQ                        |            | 2188| 730.3   | 3       |
| SAH-UBE2A-F13A   | Ac         | BSTP8RRRLBFKRLQ                        |            | 2197| 733.3   | 3       |
| SAH-UBE2A-K14A   | Ac         | BSTP8RRRLBFKRLQ                        |            | 2216| 739.7   | 3       |
| SAH-UBE2A-R15A   | Ac         | BSTP8RRRLBFKRLQ                        |            | 2188| 730.3   | 3       |
| SAH-UBE2A-L16A   | Ac         | BSTP8RRRLBFKRLQ                        |            | 2231| 744.7   | 3       |
| SAH-UBE2A-Q17A   | Ac         | BSTP8RRRLBFKRLA                       |            | 2216| 739.7   | 3       |
| SAH-UBE2A-R6E    | Ac         | BSTP8RRRLBFKRLA                       |            | 2246| 749.7   | 3, ED: 4, 6 |
| SAH-UBE2A-R7E    | Ac         | BSTP8RRRLBFKRLA                       |            | 2246| 749.7   | 3, 5, ED: 4, 6 |
| SAH-UBE2A-R8E    | Ac         | BSTP8RRRLBFKRLA                       |            | 2246| 749.7   | 3, ED: 4, 6 |
| SAH-UBE2A-K14R   | Ac         | BSTP8RRRLBFKRLA                       |            | 2301| 768.0   | ED: 2    |
| SAH-UBE2A-AAA    | Ac         | BSTP8RARARBFKRLQ                     |            | 2105| 702.6   | ED: 6    |

Ac: acetyl; X: S-pentenyl alanine; 8: R-octenyl alanine; B: norleucine; AAA: R7A/L10A/B10A

**Supplementary Table 2. Stapled Peptide Compositions.** Nomenclature, sequence compositions, masses, and use by figure for the synthesized SAH-E2<sup>h1</sup> peptides.