Supplementary Figure Legends

Supplementary Figure S1

Overlap of the identified phosphopeptides and phosphosites between 'Forward' and 'Reverse' experiments.

Venn diagrams showing overlap between 'Forward' and 'Reverse' experiments for the identified unique Class I and Class II phosphopeptides (top left panel), unique Class I phosphopeptides (top right panel), unique Class I phosphosites (bottom left panel) and quantified unique Class I phosphosites (bottom right panel).

Supplementary Figure S2

Analysis of the number of unique Class I phosphopeptides identified per fraction for the separate 'Forward' and 'Reverse' experiments.

Bar charts showing the number of unique highest scoring Class I phosphopeptides identified per fraction and the fragmentation methods by which they were identified (higher-energy collisional dissociation (HCD) in yellow, electron transfer dissociation (ETD) in blue, and collision-induced dissociation-multistage activation (CID-MSA) in purple). for the 'Forward' (top panel) and 'Reverse' (bottom panel) experiment. The cumulative amount of identified unique phosphopeptides per fraction is plotted as a grey line, using the y-axis on the right-hand side, in which a cross marks each fraction.

Supplementary Figure S3

Protein ratios are not greatly affected by 007-AM stimulation.

Scatter plot in which the $\log_2$ protein ratios after 007-AM stimulation for the 'Forward' and 'Reverse' experiments are plotted against each other. Proteins of which at least 2 unique peptides were identified and of which the relative standard deviation (RSD) of the determined ratios was ≤30% were included in the scatter plot.

Supplementary Figure S4

007-AM regulated phosphoproteins are involved in cell-cell adhesion, cell-substratum adhesion, and cell motility as shown by pathway and GO term enrichment analyses.

(A) Bar chart showing significantly enriched pathways (*P*-value ≤0.05). (B) Bar chart showing significantly enriched gene ontology (GO) terms from the biological process (BP) domain (*P*-value ≤0.05). (C) Bar chart showing significantly enriched GO terms from the cellular compartment (CC) domain (*P*-value ≤0.05). (D) Bar chart showing significantly enriched GO terms from the molecular function (MF) domain (*P*-value ≤0.05).