Supporting Information

Phosphoproteomic characterization of the signaling network resulting from activation of the chemokine receptor CCR2

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Separate Excel Files

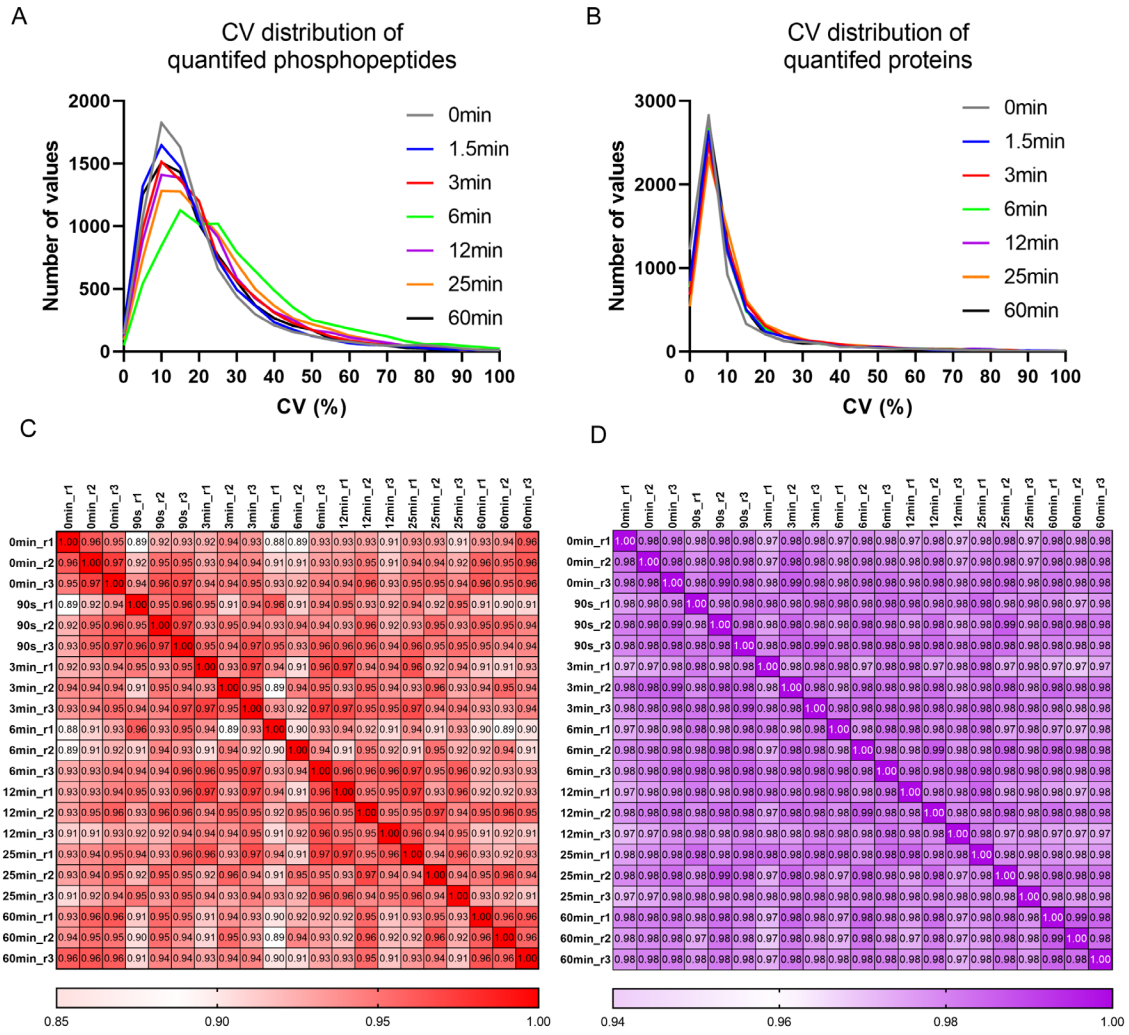

Figure S1: Coefficients of variation (CV) and Pearson correlations of the quantified phosphopeptides and proteins. (A,B) The distribution of the coefficients of variation (CVs) is shown for the quantified phosphopeptides (A) and proteins (B) within each time point (n=3). (C,D) These color-coded matrices show the Pearson correlation coefficients, which have been determined for each possible pairwise comparison within the phosphoproteomic (C) and proteomic (D) sample batch.

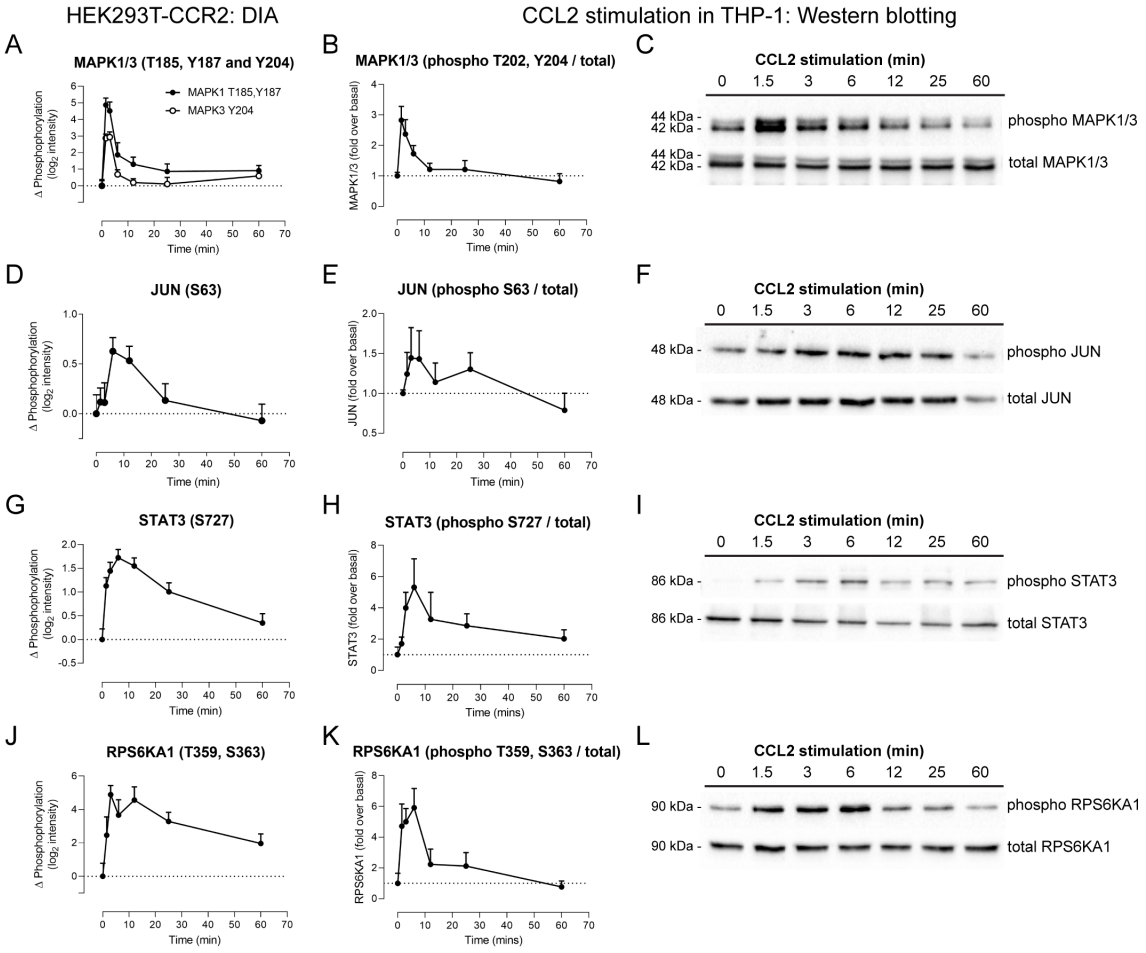

Figure S2: Phosphorylation time courses in cells that endogenously express CCR2. Phosphorylation data at the indicated sites are presented for (A-C) MAPK1/3, (B-F) JUN, (G-I) STAT3 and (J-L) RPS6KA1. (A, D, G, J) Phosphorylation time courses in FlpIn-HEK293T-CCR2 cells determined by DIA phosphoproteomics. Data represent CCL2-dependent changes in phosphopeptide intensity relative to unstimulated cells, mean ± SEM from 3 biological replicates. (B, E, H, K) Phosphorylation time courses in THP-1 cells determined by Western blotting. Data represent CCL2-dependent changes in phosphoprotein intensity relative to total protein, mean ± SEM from 3 biological replicates. (C, F, I, L) Representative Western blots. Molecular weight markers have been cropped out; predicted molecular weights of the proteins are indicated.

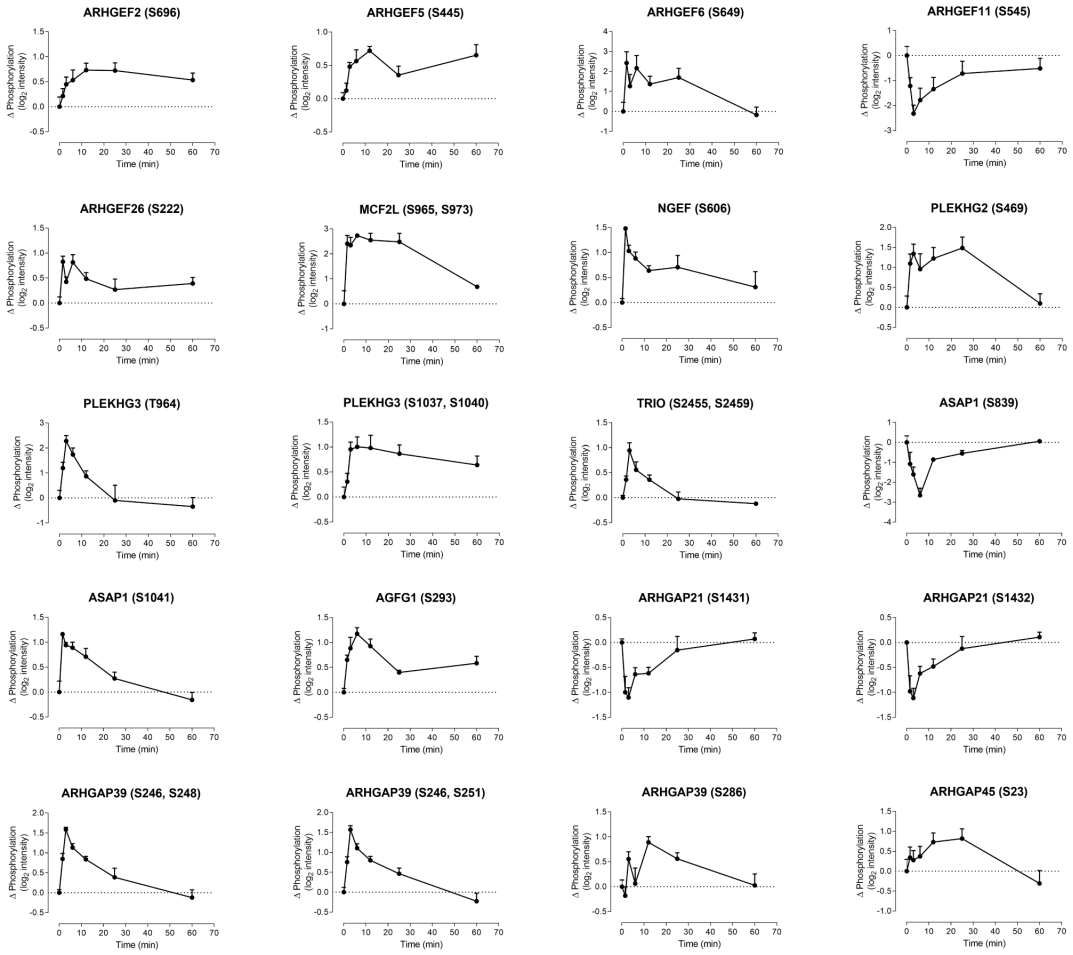

Figure S3: Phosphorylation time courses in CCR2-expressing cells for a selection of guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Data represent CCL2-dependent changes in phosphopeptide intensity relative to unstimulated cells, mean ± SEM from 3 biological replicates.

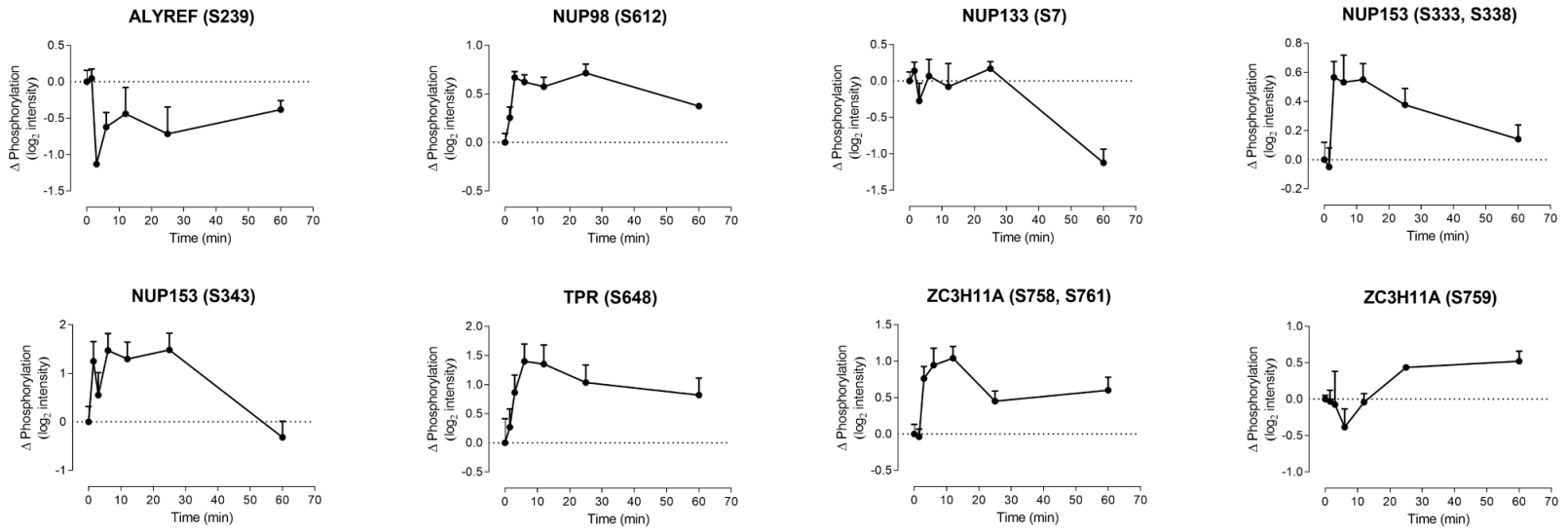

Figure S4: Phosphorylation time courses in CCR2-expressing cells for a selection of nuclear-related proteins. Data represent CCL2-dependent changes in phosphopeptide intensity relative to unstimulated cells, mean ± SEM from 3 biological replicates.

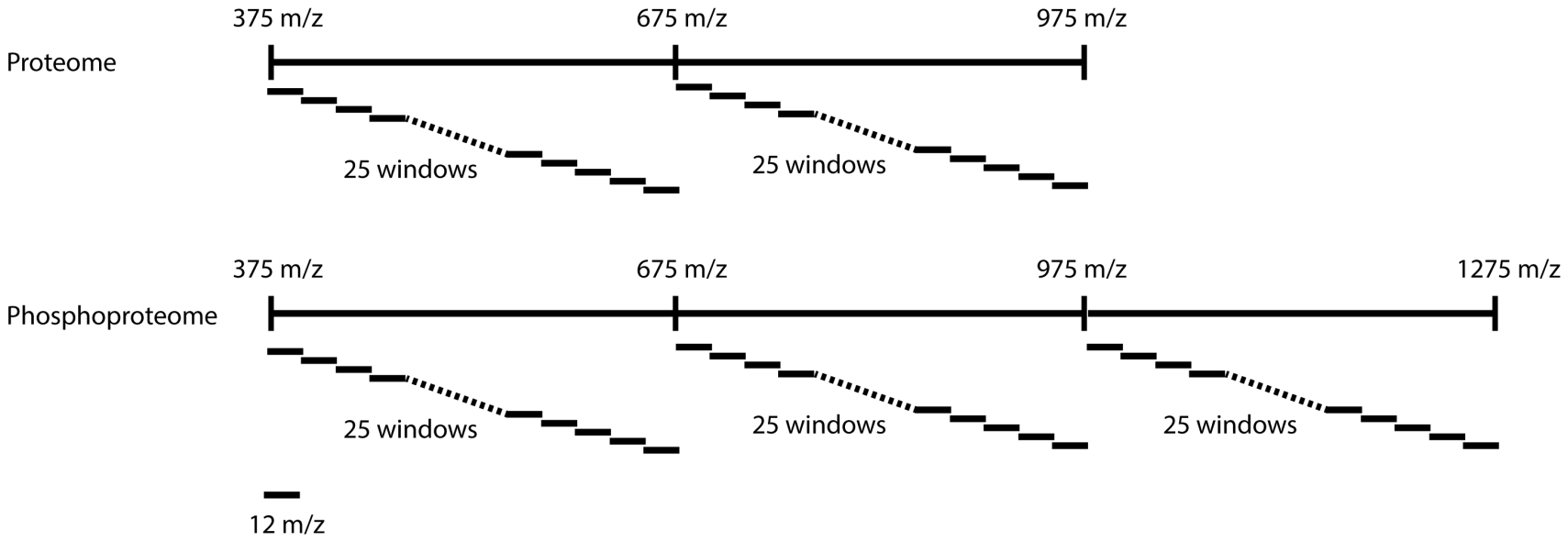

Figure S5: A schematic illustration of the DIA strategy used for proteome quantification (top panel) and phosphoproteome quantification (bottom panel). In each DIA run, 25 windows with an isolation width of 12 m/z have been acquired. To cover a m/z range of 375 - 975 m/z for the global proteomic analyses (top panel) and 375 - 1275 m/z for the phosphoproteomic analyses (bottom panel), each proteomic sample was injected twice and each phosphoproteomic sample three times.