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Supplemental information

Exploring temporal and sex-linked dysregulation in Alzheimer disease phosphoproteome

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Supplementary Figures

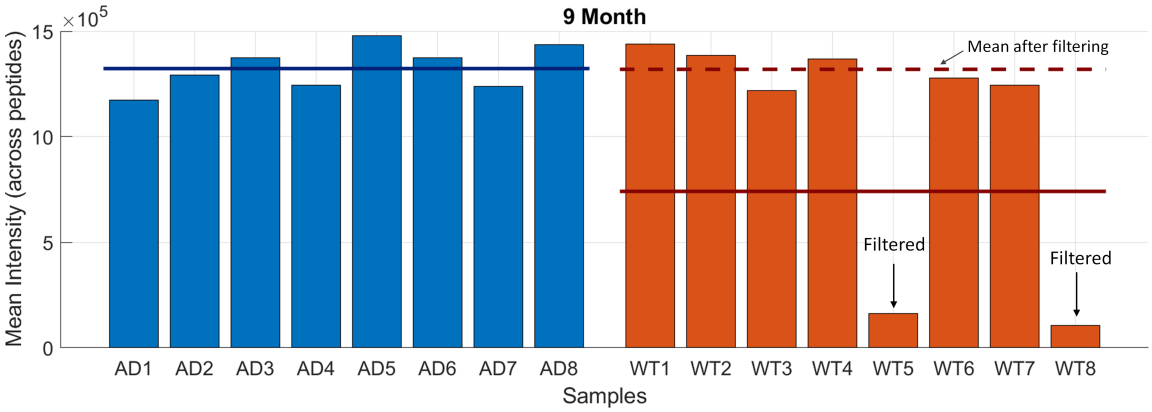

Figure S1: **Mean intensities across all phosphopeptides in the 9 month time point, related to STAR methods.** Samples named as AD1 to AD8 are in the 5XFAD group, and WT1 to WT8 are in the control (wildtype) group. Two WT samples with abnormally low intensities are marked on the plot. The solid lines indicate the mean values across samples. The dashed line on the WT group indicates the mean value after the two samples are filtered out.

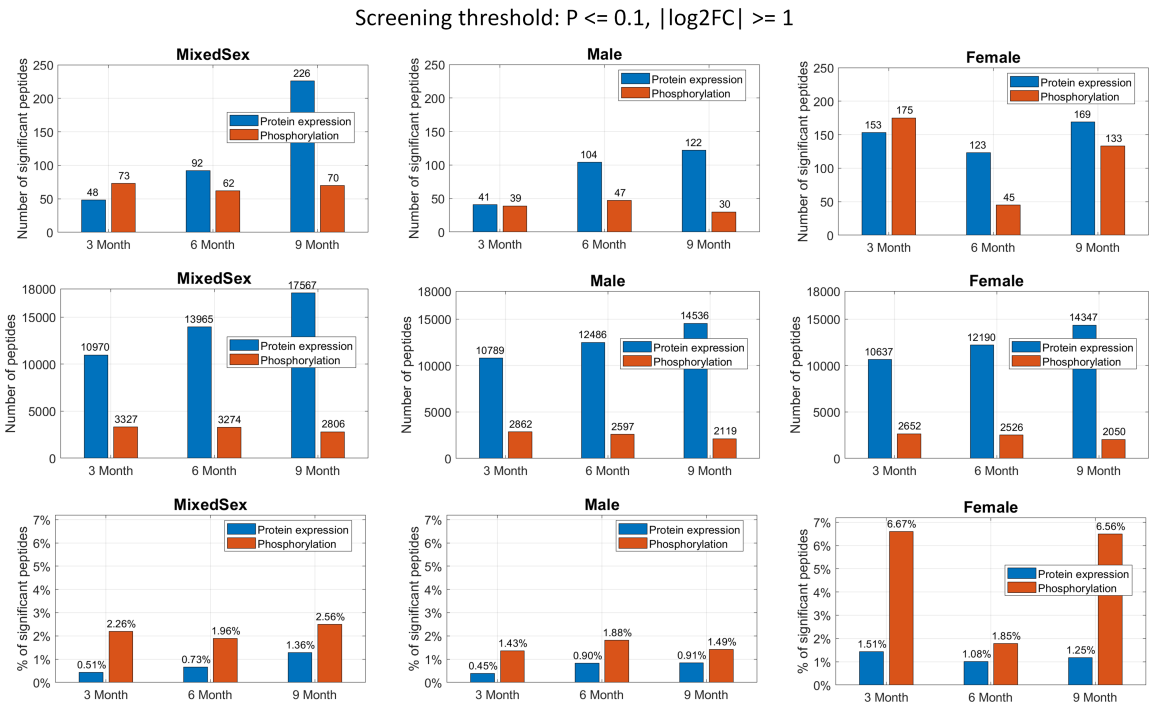

Figure S2: **Prevalence of phosphorylated or expressed peptides in Alzheimer's disease 5XFAD model based on different time points and sex, related to STAR methods.** (Top panels) The number of peptides that pass the screening threshold ($p \leq 0.1$ and $0.5 \geq FC \geq 2$) for phosphorylation or expression for 3/6/9 month data. Left to right, each panel corresponds to a different analysis based on the sex of the samples: MixedSex, Male, and Female. (Middle panels) Total number of identified peptides. (Bottom panels) Percentage of the peptides that pass the screening threshold.

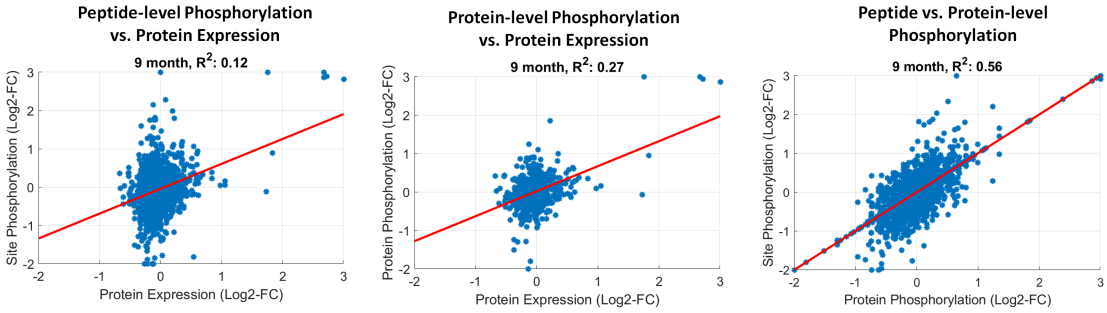

Figure S3: **Association and complementarity of phosphorylation information to protein expression in Alzheimer's disease 5XFAD model phospho-proteome, related to STAR methods.** (Left) Scatter plot indicating the association between peptide-level phosphorylation and protein expression for the 9 month data. Each point represents a phospho-peptide and the x and y axes respectively indicate the log2 fold change values for protein expression and mean phosphorylation for the corresponding protein. (Left) Scatter plot indicating the association between phosphorylation and protein expression for the 9 month data. Each point represents a protein and the x and y axes respectively indicate log2 fold change values for protein expression and mean phosphorylation for the protein. (Right) Scatter plot indicating the association between phosphosite and protein-wise phosphorylation for the 9 month data. Each point represents a phosphosite and the x and y axes respectively indicate log2 fold change values for phosphorylation of the site and the mean phosphorylation for the corresponding protein. (All panels) The red line indicates the best fit line and the squared correlation (R^2) values for all timepoints are specified below the plots.

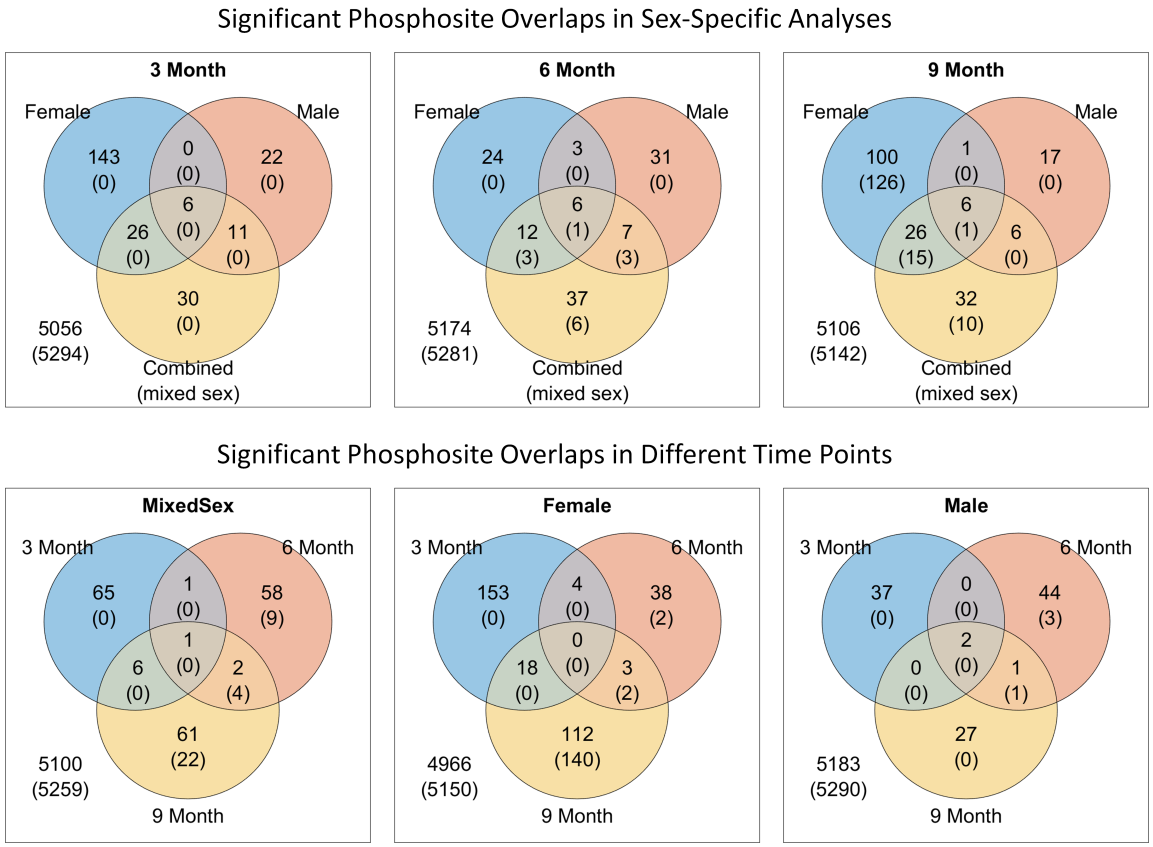

Figure S4: **Venn diagrams of the number of significant phosphosites for sex-specific analysis, related to Figure 1.** The numbers in the venn diagram indicate the number of phosphosites that pass the screening ($p \leq 0.1$ and $0.5 \geq FC \geq 2$). The numbers in parentheses indicate the number of significant phosphosites that pass FDR$\leq$0.1. Top panels correspond to different time points (3/6/9 months), and bottom panels represent the type of sex-specific analysis (MixedSex/Female/Male).

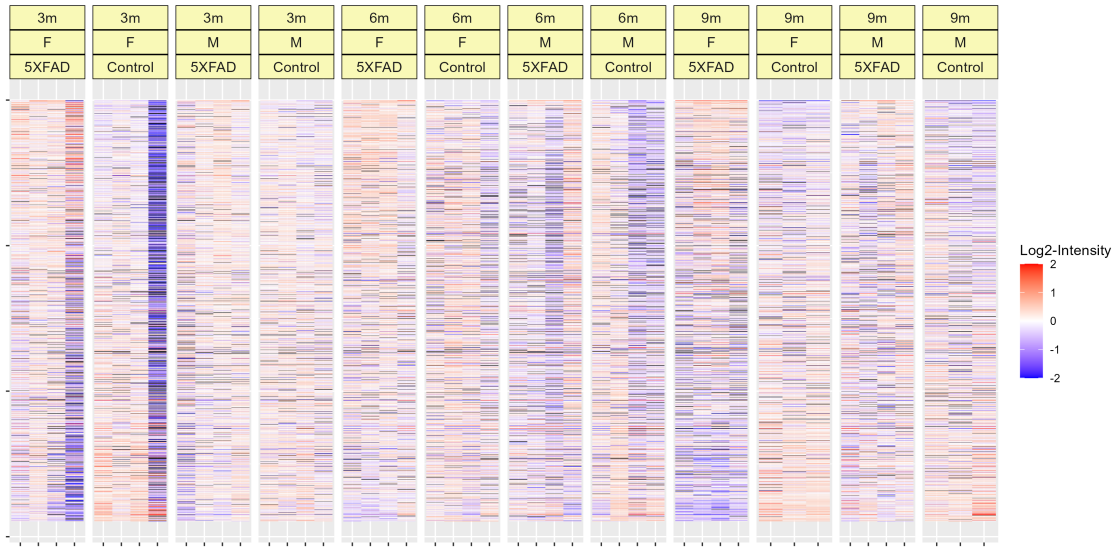

Figure S5: **Heatmap showing genotype and sex variability within the phosphoproteome for each time point of 5XFAD model, related to STAR methods.**

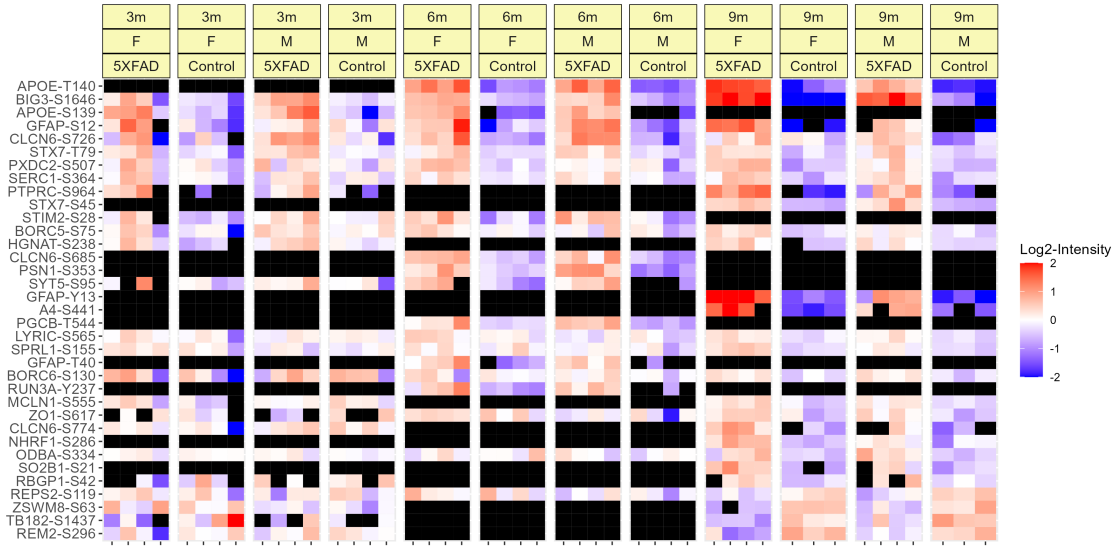

Figure S6: **Heatmap showing genotype and sex variability for phosphosites identified as differentially phosphorylated with FDR≤0.1 for each time point of 5XFAD model, related to Figure 1.**

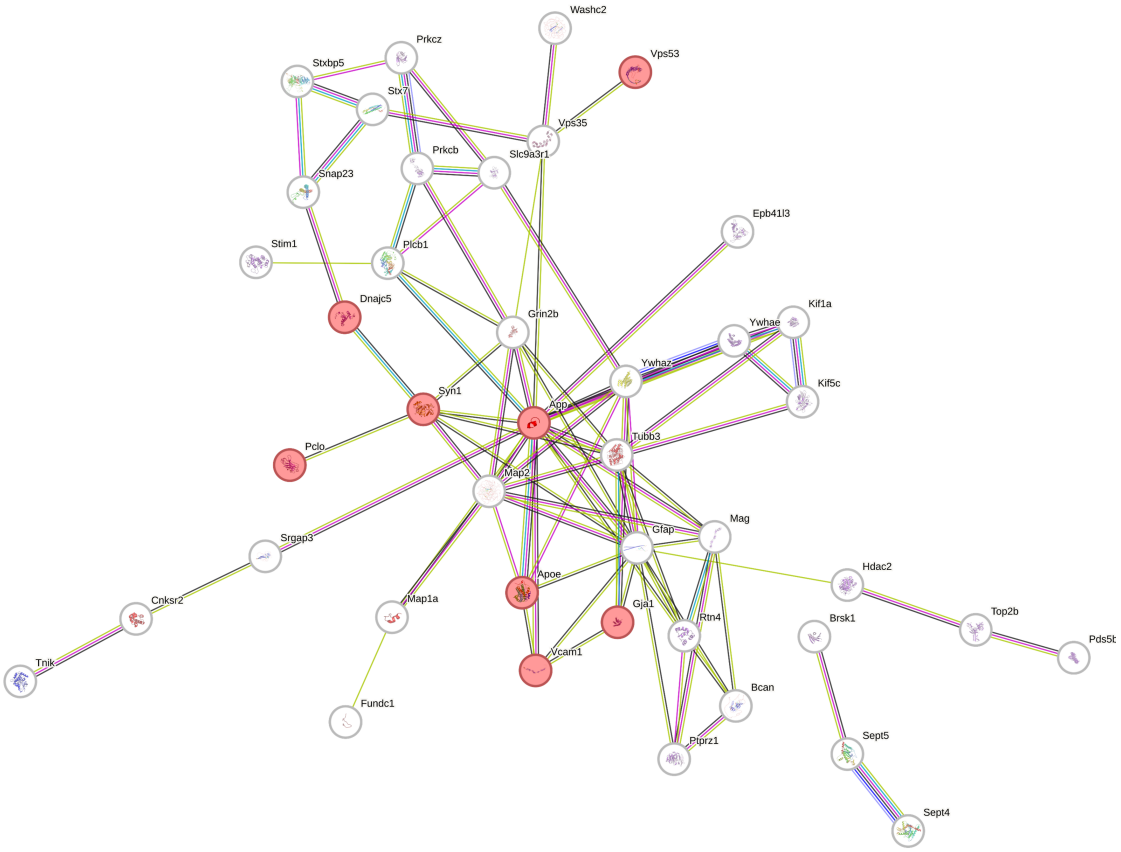

Figure S7: **STRING network analysis based on the top phosphorylated proteins in 9 month female group, related to Figure 1.** Golgi apparatus related proteins are highlighted in red.

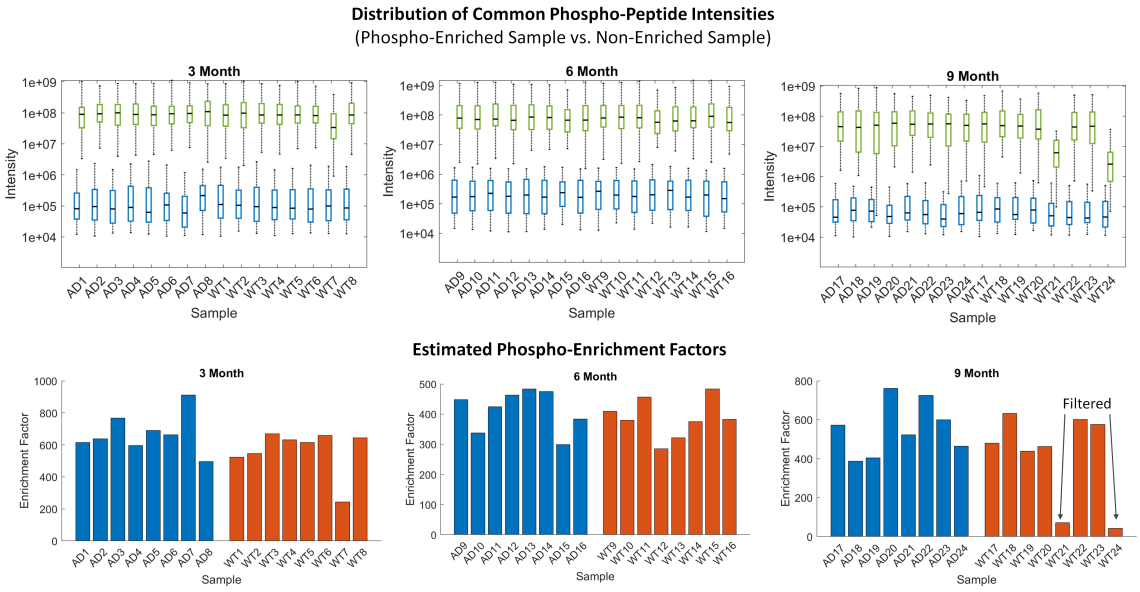

Figure S8: **Phospho-enriched vs. unenriched sample intensities, related to STAR methods.** (Top panels) Box plots indicating the distribution of the top phospho-peptide intensities for common proteins identified in phospho-enriched and unenriched samples. Each panel corresponds to a different time point. In each panel, green boxes on the top side correspond to phospho-enriched samples and blue boxes represent unenriched samples. (Bottom panels) Estimated phosphorylation enrichment factors based on the mean difference in the distribution of phospho-enriched samples and unenriched samples.

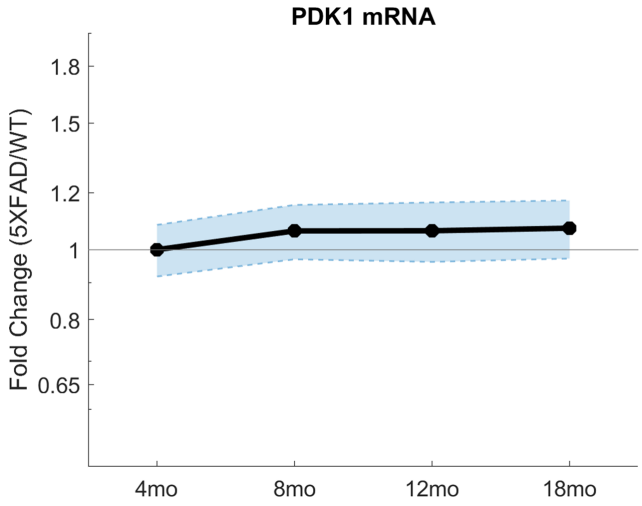

Figure S9: **mRNA expression of PDK1 for various time points in a longitudinal 5XFAD transcriptomic study [Forner, 2021], related to Figure 4.** The blue shaded area represents the 95% confidence interval for each time point.