Supplementary Text

Statistical inference at protein level

To identify phosphopeptides that are significant, we perform moderated t-test, comparing the 5XFAD samples with the wildtype (WT) samples. Let $q[i]$ be the resultant log2 fold change, and $\sigma[i]$, $df[i]$ be the corresponding standard error and degrees of freedom obtained from moderated t-test for peptide i.

To identify potential biomarkers based on phosphorylation data that are consistent across time points, we perform the analysis at the protein level. For this purpose, we first compute the mean log-fold changes $q_p[j]$ for each protein j:

$$q_p[j] = \frac{\sum_{i \in \mathcal{V}_j} q[i]}{|\mathcal{V}_j|} \tag{1}$$

where $\mathcal{V}_j$ denotes the set of phosphopeptides corresponding to protein j.

To estimate the pooled standard error $\sigma_p[j]$ and the corresponding degrees of freedom $df_p[j]$ in the estimation of the mean log-fold changes for each protein j, we use the Satterthwaite approximation:

$$\begin{aligned} \sigma_p[j] &= \frac{\sqrt{\sum_{i \in \mathcal{V}_j} \sigma^2[i]}}{|\mathcal{V}_j|} \\ df_p[j] &= \frac{\left(\sum_{i \in \mathcal{V}_j} \sigma^2[i]\right)^2}{\sum_{i \in \mathcal{V}_j} \left(\frac{\sigma^4[i]}{df[i]}\right)} \end{aligned} \tag{2}$$

Based on these estimations, to compute the significance of a protein j, a t-test is performed with the t-statistic $t_p[j]$:

$$t_p[j] = \frac{q_p[j]}{\sigma_p[j]} \tag{3}$$

which follows a t-distribution with $df_p[j]$ degrees of freedom under the null hypothesis.

Statistical tests to identify sex differences

Let $q_{\text{male}}[i]$ denote the log2 fold change, and $\sigma_{\text{male}}[i]$, $df_{\text{male}}[i]$ denote the corresponding standard error and degrees of freedom for obtained from t-test for the male group for phosphosite i. Similarly, let $q_{\text{female}}[i]$, $\sigma_{\text{female}}[i]$, $df_{\text{female}}[i]$ denote the resulting values for the female group. The log2 fold change for the sex difference between the two groups is given by:

$$q_{\text{sexdif}}[i] = q_{\text{female}}[i] - q_{\text{male}}[i] \tag{4}$$

Since the male and female groups consist of independent set of samples, with the equal variance assumption, the corresponding standard error and degrees of freedom for $q_{\text{sexdif}}[i]$ is as follows:

$$\begin{aligned} \sigma_{\text{sexdif}}[i] &= \sqrt{\frac{df_{\text{female}}[i]\sigma^2_{\text{female}}[i] + df_{\text{male}}[i]\sigma^2_{\text{male}}[i]}{df_{\text{sexdif}}[i]}} \\ df_{\text{sexdif}}[i] &= df_{\text{female}}[i] + df_{\text{male}}[i] \end{aligned} \tag{5}$$

After this step, similar to the individual tests done before, we enhance the degrees of freedom for individual variances by applying a moderated t-test, utilizing the *squeezeVar* function from the *limma* package in R to shrink sample variances towards a common value. Based on these, the p-values and statistical significance are computed as usual from the t-distribution.

Note that, this test corresponds to the following linear model where sex is used as a covariate:

$$\begin{aligned} \backsim \ & \left(\boldsymbol{x}_{\text{female}}^{\text{5XFAD}}[i] - \boldsymbol{x}_{\text{female}}^{\text{Control}}[i]\right) - \left(\boldsymbol{x}_{\text{male}}^{\text{5XFAD}}[i] - \boldsymbol{x}_{\text{male}}^{\text{Control}}[i]\right) \\ & = \boldsymbol{x}_{\text{female}}^{\text{5XFAD}}[i] - \boldsymbol{x}_{\text{female}}^{\text{Control}}[i] - \boldsymbol{x}_{\text{male}}^{\text{5XFAD}}[i] + \boldsymbol{x}_{\text{male}}^{\text{Control}}[i] \end{aligned} \tag{6}$$

where $\boldsymbol{x}$ denote the mean log2 intensities for the samples in the corresponding group.

Consistency analysis to identify protein biomarkers

To assess consistency across time points, we introduce a simple statistic called the consistency score, which combines the log2-fold change results of individual time points. This score represents the total log2-fold change of proteins across time points, considering any missing values as log2-fold change of 0.

Let $\mathcal{T} = \{3 \text{ month}, 6 \text{ month}, 9 \text{ month}\}$ denote the set of time points, and let $q_p^{(t)}[j]$, $\sigma_p^{(t)}[j]$, and $df_p^{(t)}[j]$ respectively denote the log-fold changes, standard error, and the degrees of freedom for protein j corresponding to the analysis performed for time point $t \in \mathcal{T}$. Based on these, we compute the consistency score $q_c[j]$ (i.e., total log fold change) for protein j as follows:

$$q_c[j] = \sum_{t \in \mathcal{T}} q_p^{(t)}[j] \tag{7}$$

The corresponding standard error $\sigma_c[j]$ and the degrees of freedom $df_c[j]$ for the consistency score are estimated by the Satterthwaite approximation:

$$\begin{aligned} \sigma_c[j] &= \sqrt{\sum_{t \in \mathcal{T}} \left(\sigma_p^{(t)}[j]\right)^2} \\ df_p[j] &= \frac{\left(\sum_{t \in \mathcal{T}} \left(\sigma_p^{(t)}[j]\right)^2\right)^2}{\sum_{t \in \mathcal{T}} \left(\frac{\left(\sigma_p^{(t)}[i]\right)^4}{df_p^{(t)}[i]}\right)} \end{aligned} \tag{8}$$

Note that, if a protein j have a missing data at time point t, we consider it to have $q_p^{(t)}[j] = 0$, $\sigma_p^{(t)}[j] = 0$ and $df_p^{(t)}[j] = \infty$ during the computation of the consistency score.

Statistical inference at pathway level

To understand the biological pathways and networks impacted by the observed phosphoproteome changes, we performed a quantitative pathway enrichment analysis based on the mean phosphorylation (log2-FC) of proteins.

Let $\mathcal{P}_k$ denote the set of proteins corresponding to pathway k with non-missing data (i.e., each protein having at least one phosphopeptide identified in our dataset). To estimate the enrichment of pathway k, we compute the mean log fold change $q_e[k]$ for each protein in $\mathcal{P}_k$ as follows:

$$q_e[k] = \frac{\sum_{j \in \mathcal{P}_k} q_p[j]}{|\mathcal{P}_k|} \tag{9}$$

The corresponding standard error $\sigma_c[j]$ and the degrees of freedom $df_c[j]$ for the consistency score are estimated by the Satterthwaite approximation:

$$\begin{aligned} \sigma_e[k] &= \frac{\sqrt{\sum_{j \in \mathcal{P}_k} \sigma_p^2[j]}}{|\mathcal{P}_k|} \\ df_e[k] &= \frac{\left(\sum_{j \in \mathcal{P}_k} \sigma_p^2[j]\right)^2}{\sum_{j \in \mathcal{P}_k} \left(\frac{\sigma_p^4[j]}{df_p[j]}\right)} \end{aligned} \tag{10}$$

Similar to the protein analysis, the statistical significance of a pathway k is then assessed with a t-test, based on the t-statistic:

$$t_e[k] = \frac{q_e[k]}{\sigma_e[k]} \tag{11}$$

which follows a t-distribution with $df_e[k]$ degrees of freedom under the null hypothesis.