

---

# **Supporting Information:**

## **Mouse primary T cell phosphotyrosine proteomics**

### **enabled by BOOST**

Xien Yu Chua<sup>1</sup>, Kenneth P. Callahan<sup>2</sup>, Alijah A. Griffith<sup>2</sup>, Tobias Hildebrandt<sup>2</sup>,  
Guoping Fu<sup>3</sup>, Mengzhou Hu<sup>1</sup>, Renren Wen<sup>3</sup>, Arthur R. Salomon<sup>2,\*</sup>

*1 Department of Molecular Pharmacology, Physiology & Biotechnology, Brown University, Providence, RI, 02912*

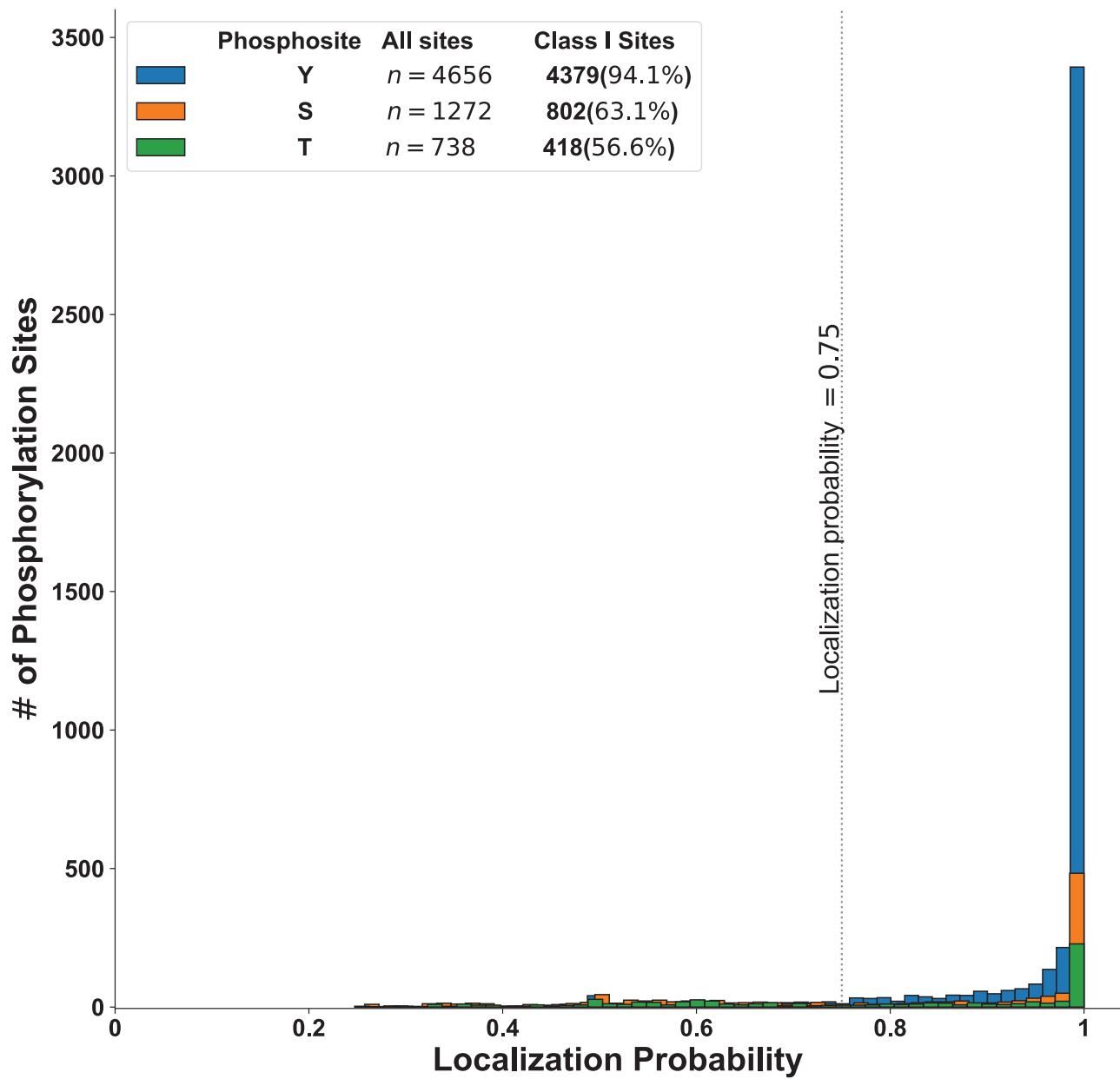
*2 Department of Molecular Biology, Cell Biology & Biochemistry, Brown University, Providence, RI, 02912*

*3 Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI, 53226*  
*\* Corresponding Author\**

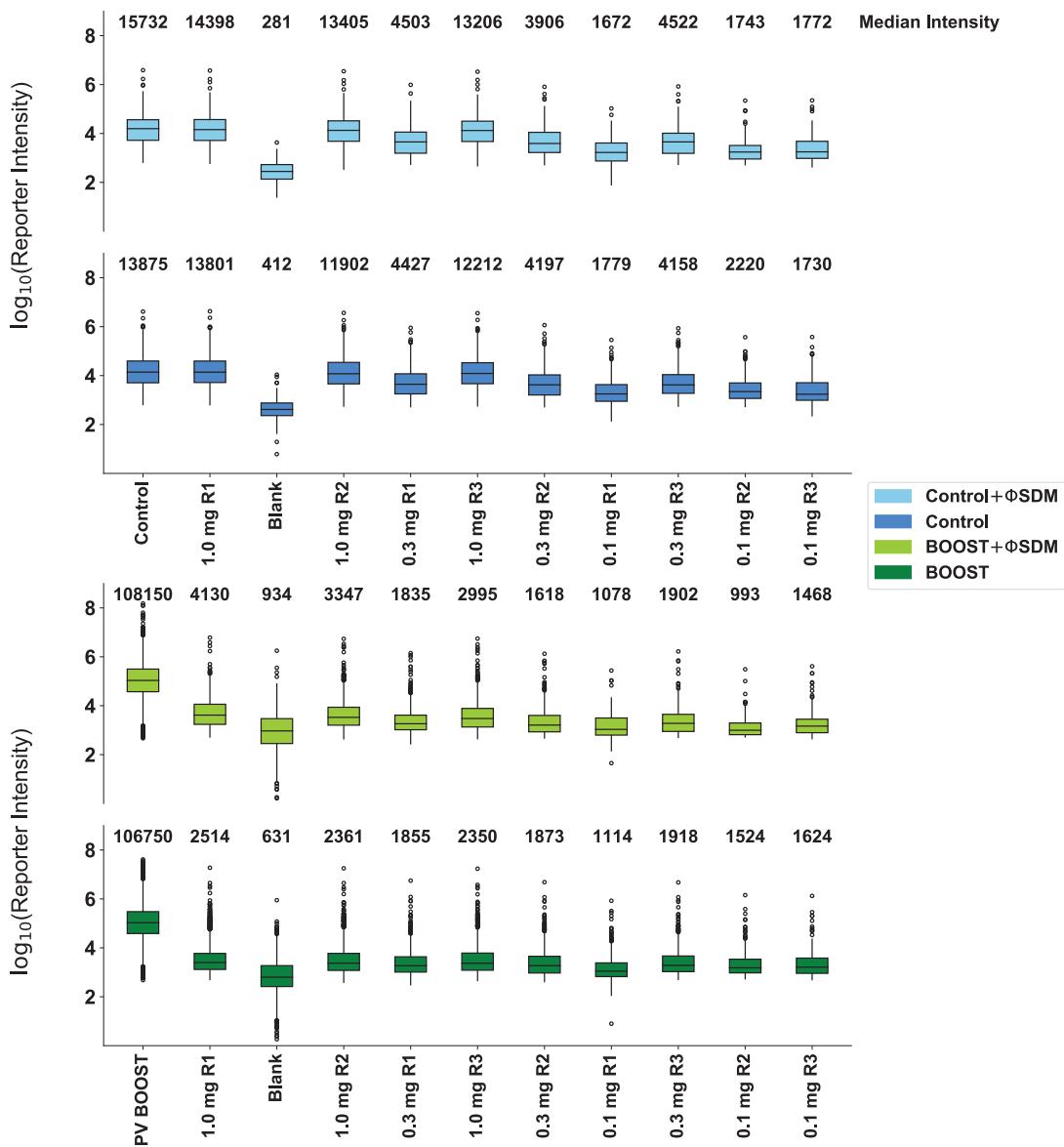
E-mail: art@drsalomon.com

## Table Of Contents

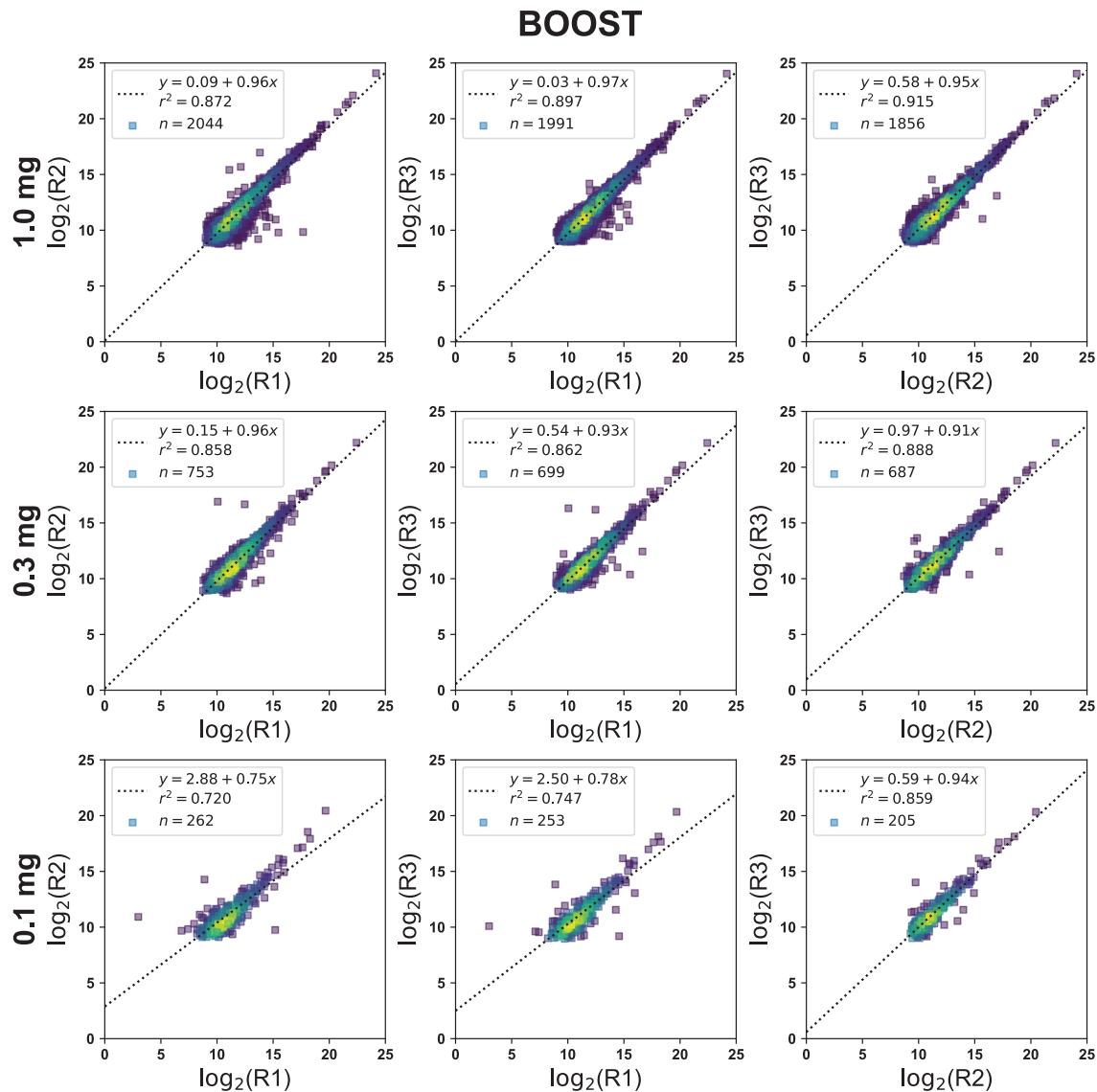
- Supporting Figure 1:** Histogram of all PSMs containing a phosphorylated amino acid in all conditions binned by localization probability
- Supporting Figure 2:**  $\log_{10}$  transformed reporter intensity box-and-whisker plots
- Supporting Figure 3:** Pairwise replicate comparisons of unique peptides identified in BOOST when  $\Phi$ SDM is disabled
- Supporting Figure 4:** Pairwise replicate comparisons of unique peptides identified in BOOST when  $\Phi$ SDM is enabled
- Supporting Figure 5:** Pairwise replicate comparisons of unique peptides identified in 1.0 mg Control when  $\Phi$ SDM is disabled
- Supporting Figure 6:** Pairwise replicate comparisons of unique peptides identified in 1.0 mg Control when  $\Phi$ SDM is enabled
- Supporting Figure 7:** Venn Diagram and Volcano Plots for BOOST and 1.0 mg Control when  $\Phi$ SDM is disabled
- Supporting Figure 8:** Venn Diagram and Volcano Plots for BOOST and 1.0 mg Control when  $\Phi$ SDM is enabled
- Supporting Figure 9:** Venn Diagrams for BOOST conditions (with and without  $\Phi$ SDM) and 1.0 mg Control (with and without  $\Phi$ SDM)
- Supporting Figure 10:** Cumulative distributions of unique pTyr peptides from BOOST experiments (with and without  $\Phi$ SDM)



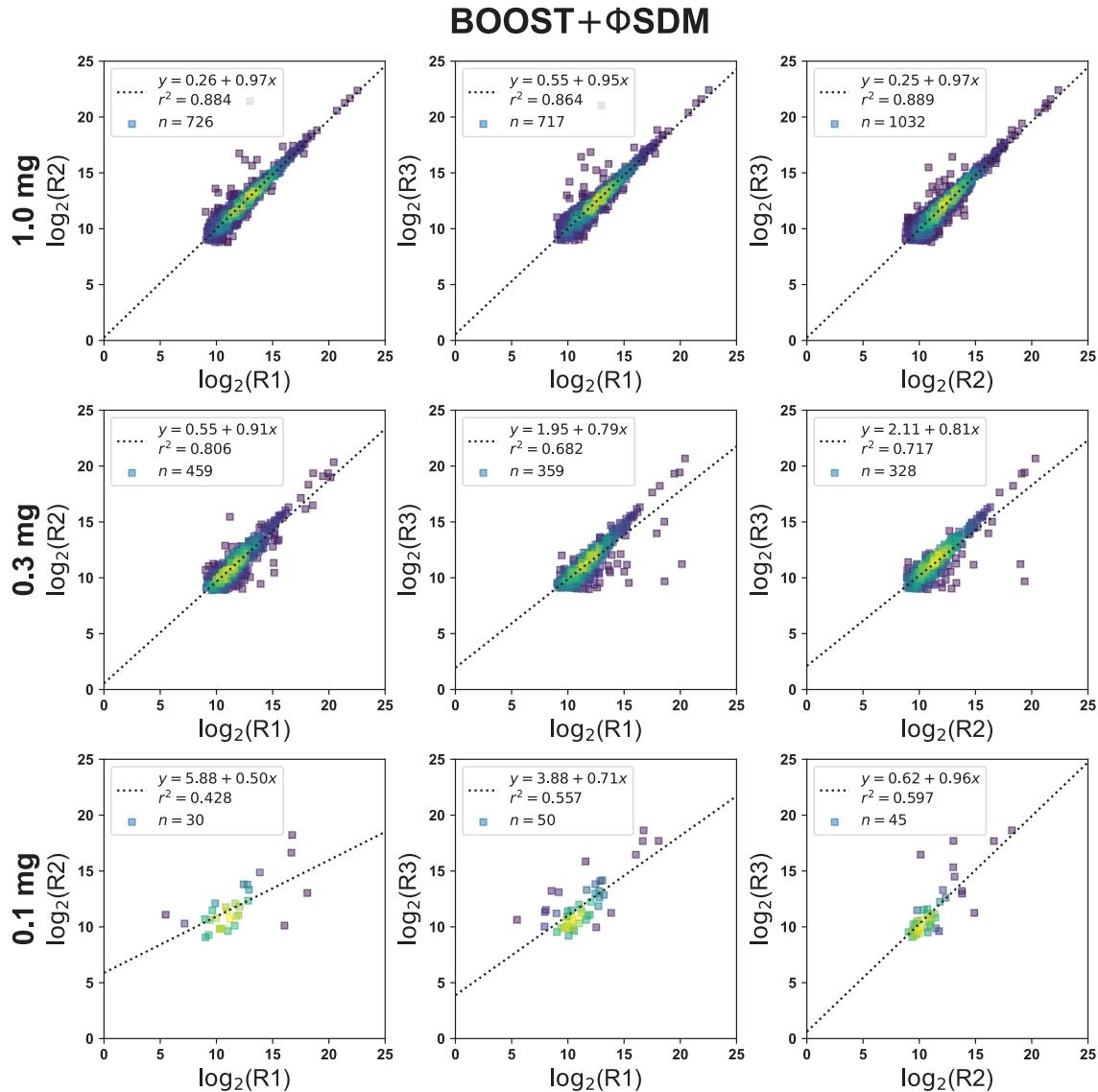
Supporting Figure 1: A histogram with depicting all PSMs from all experiments containing at least one phosphorylated serine (S), threonine (T), or tyrosine (Y) amino acid as a function of localization probability ( $n_{bins} = 75$ ). The total number and number of Class I (localization probability  $> 0.75$ ) phosphorylation sites for each amino acid are noted in the Figure Legend.



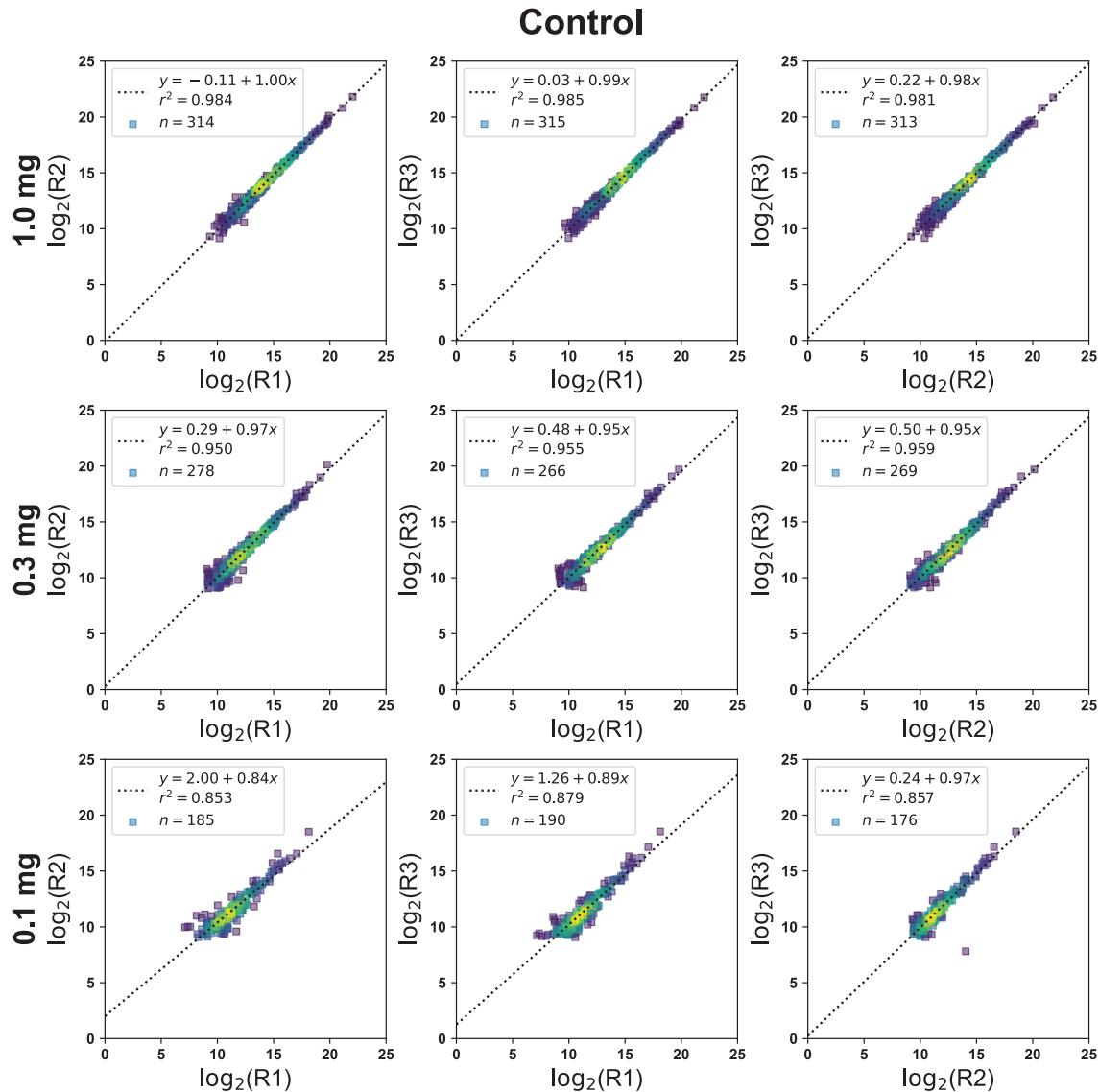
Supporting Figure 2: Box-and-whisker plots showing the  $\log_{10}$  transformed reporter intensities for each TMT mix and each condition. Non-transformed, median intensities are displayed above each box-and-whisker plot.



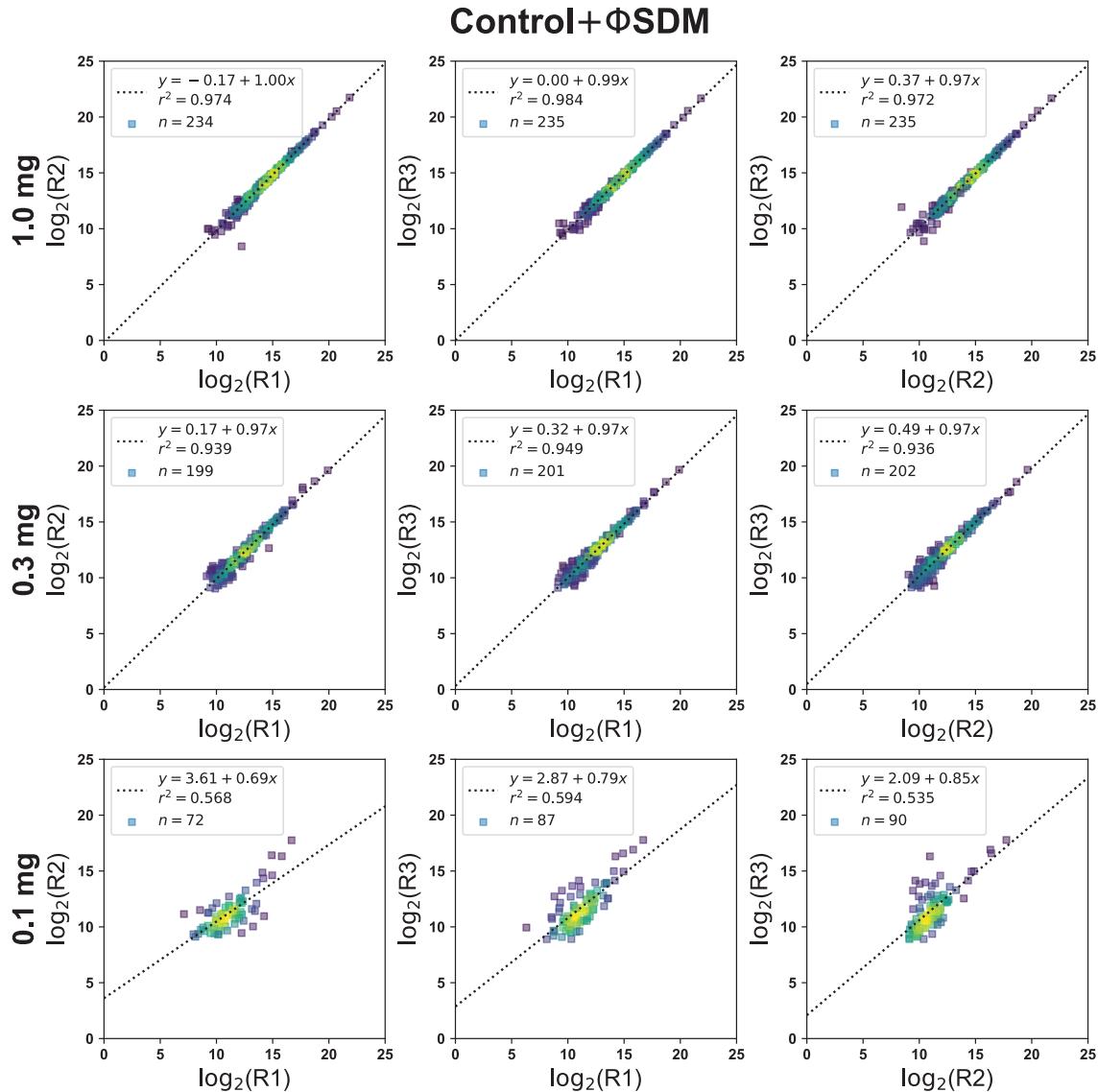
Supporting Figure 3: Replicate reproducibility is stable when ΦSDM is disabled for low protein input samples in the pervanadate BOOST condition. Evaluation of replicate reproducibility in the BOOST experiment (with ΦSDM disabled) using pairwise comparisons of  $\log_2$  transformed abundances for phosphopeptides with the same charge state and assigned sequence. For each comparison, we show the line of best fit as determined by simple least-squares regression, the  $r^2$  value as an estimate of the quality of the fitted line, and the total number of points ( $n$ ) in each comparison.



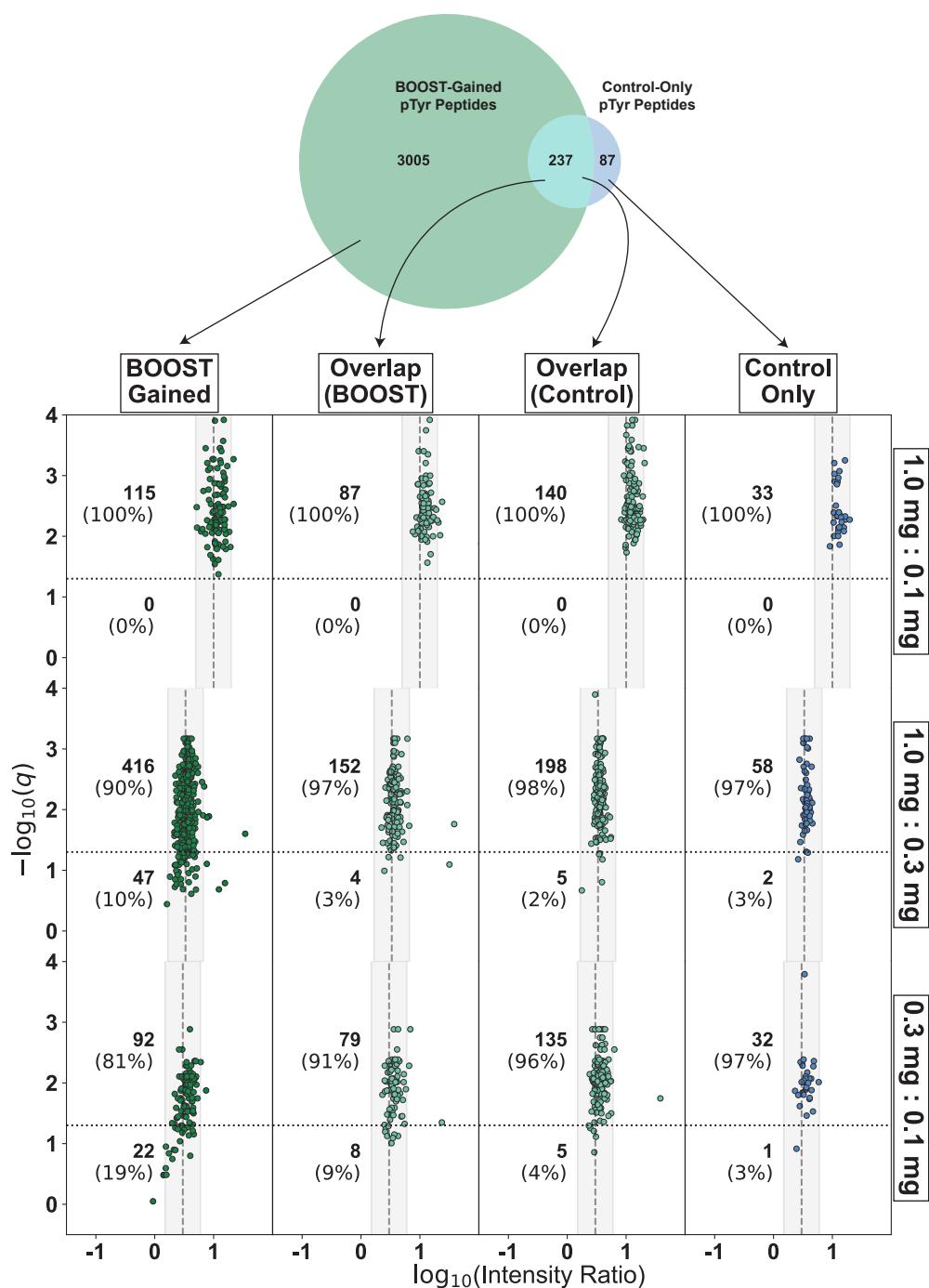
Supporting Figure 4: Replicate reproducibility is severely degraded when ΦSDM is enabled for low protein input samples in the pervanadate BOOST condition. Evaluation of replicate reproducibility in the BOOST experiment (with ΦSDM enabled) using pairwise comparisons of  $\log_2$  transformed abundances for phosphopeptides with the same charge state and assigned sequence. For each comparison, we show the line of best fit as determined by simple least-squares regression, the  $r^2$  value as an estimate of the quality of the fitted line, and the total number of points ( $n$ ) in each comparison.



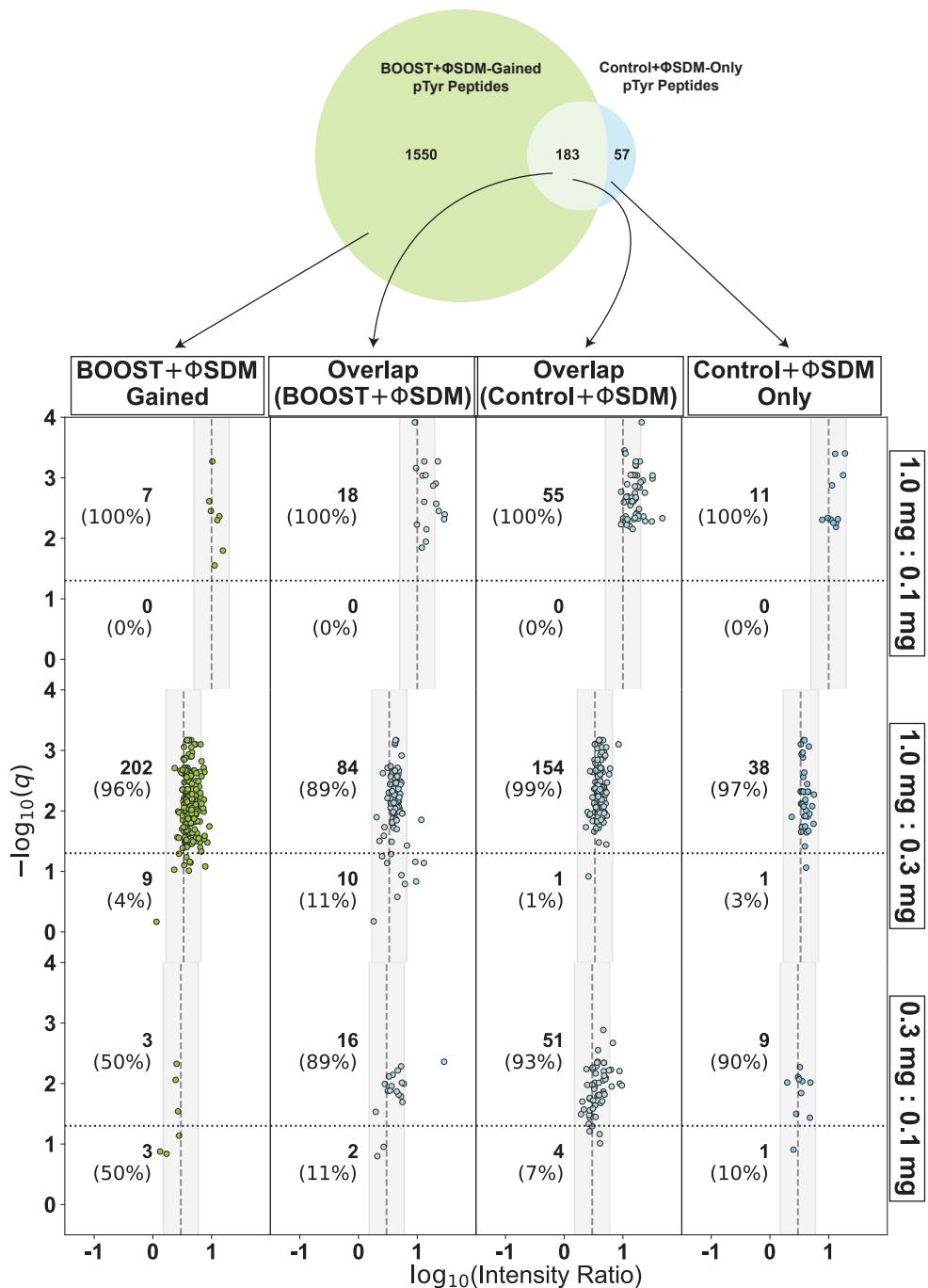
Supporting Figure 5: Replicate reproducibility is stable when ΦSDM is disabled for low protein input samples in the 1.0 mg Control condition. Evaluation of replicate reproducibility in the 1.0 mg Control experiment (with ΦSDM disabled) using pairwise comparisons of  $\log_2$  transformed abundances for phosphopeptides with the same charge state and assigned sequence. For each comparison, we show the line of best fit as determined by simple least-squares regression, the  $r^2$  value as an estimate of the quality of the fitted line, and the total number of points ( $n$ ) in each comparison.



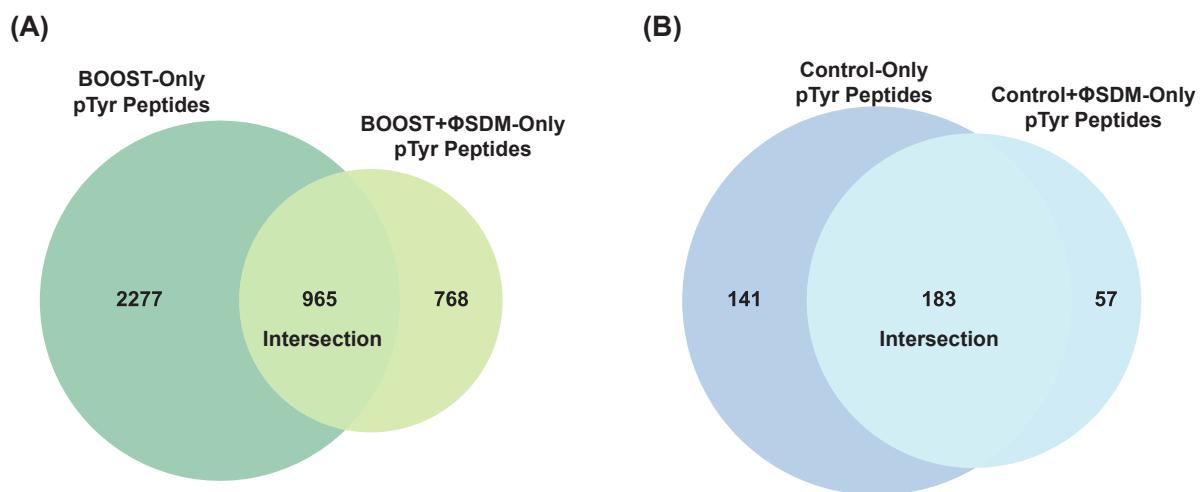
Supporting Figure 6: Replicate reproducibility is degraded when ΦSDM is enabled for low protein input samples in the 1.0 mg Control condition. Evaluation of replicate reproducibility in the 1.0 mg experiment (with ΦSDM enabled) using pairwise comparisons of  $\log_2$  transformed abundances for phosphopeptides with the same charge state and assigned sequence. For each comparison, we show the line of best fit as determined by simple least-squares regression, the  $r^2$  value as an estimate of the quality of the fitted line, and the total number of points ( $n$ ) in each comparison.



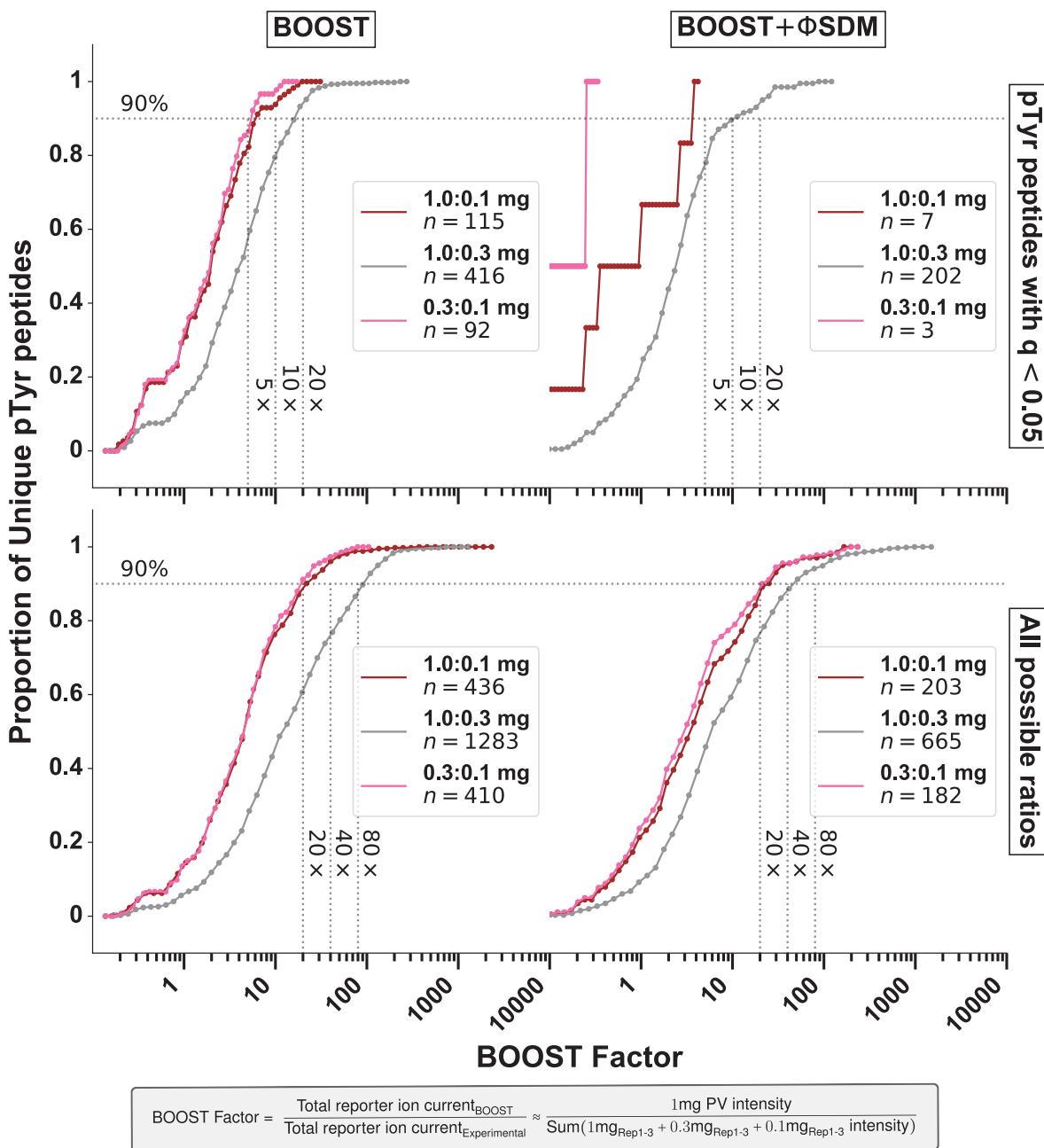
Supporting Figure 7: With  $\Phi$ SDM disabled, the pervanadate BOOST channel dramatically increases the number of unique pTyr peptides observed as compared to a 1.0 mg Control channel. A Venn diagram showing the overlap of unique pTyr peptides between the BOOST and 1.0 mg Control experiments (with  $\Phi$ SDM disabled). Volcano plots show  $-\log_{10}(q\text{-value})$  as a function of  $\log_{10}(\text{Intensity Ratio})$  for unique pTyr peptides from groups shown in the Venn diagram. For the overlapping section, volcano plots were created using data from both the BOOST experiment and the control experiment acquired with  $\Phi$ SDM disabled.



Supporting Figure 8: The pervanadate BOOST channel increases the number of unique pTyr peptides observed when ΦSDM is enabled, although few peptides are observed in low abundance samples. A Venn diagram showing the overlap of unique pTyr peptides between the BOOST+ΦSDM and 1.0 mg Control+ΦSDM experiments. Volcano plots show  $-\log_{10}(q\text{-value})$  as a function of  $\log_{10}(\text{Intensity Ratio})$  for unique pTyr peptides from groups shown in the Venn diagram. For the overlapping section, volcano plots were created using data from both the BOOST experiment and the control experiment acquired with ΦSDM enabled.



Supporting Figure 9: Enabling  $\Phi$ SDM results in lower yield in both pervanadate BOOST and 1.0 mg Control conditions. Venn diagrams showing the number of unique pTyr peptides observed when  $\Phi$ SDM is enabled or disabled using (A) pervanadate BOOST samples, and (B) 1.0 mg Control samples.



Supporting Figure 10: Enabling ΦSDM decreases quantitation depth, particularly in low abundance samples. Cumulative distribution of BOOST factors for unique pTyr peptides identified in the pervanadate BOOST experiments with ΦSDM disabled or with ΦSDM enabled for pTyr peptides with a statistically significant ratio ( $q < 0.05$ ) or for all calculable ratios. For each cumulative distribution, the range of BOOST factors are split into 50 bins of equal size on a  $\log_{10}$  scale.