

Supplementary Material

Phosphoproteome Analysis Using Two-Dimensional Electrophoresis Coupled with Chemical Dephosphorylation

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Supplementary Tables S1–3

Supplementary Figure S1

Table S1

Mean volume of *PR* (\pm SE) and 95% CI interval for 2-DE spots with statistically significant differential phosphorylation rate (*PR*) between HF-P and Pro-Q DPS methods to evaluate phosphorylation from LT muscle meat of Rubia Gallega (RG).

| Spot code ¹ | <i>PR</i> _{HF-P} | | <i>PR</i> _{ProQ-DPS} | | <i>P</i> -value ⁴ |
|------------------------|------------------------------|--|-------------------------------|--|------------------------------|
| | Volume \pm SE ² | 95% bootstrap CI (CL, CU) ³ | Volume \pm SE ² | 95% bootstrap CI (CL, CU) ³ | |
| 1 | 100 \pm 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 2 | 100 \pm 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 3 | 100 \pm 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 4 | 100 \pm 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 5 | 52 \pm 15.0 | 33.2, 69.9 | 0 | 0, 0 | <0.05 |
| 6 | 47 \pm 13.0 | 22.5, 68.8 | 0 | 0, 0 | <0.05 |
| 7 | 100 \pm 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 8 | 100 \pm 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |

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|----|---------------|----------|--------------|------------|-------|
| 9 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 10 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 11 | 100 ± 0.0 | 100, 100 | 13 ± 3.0 | 0.07, 0.18 | <0.05 |
| 12 | 100 ± 0.0 | 100, 100 | 10 ± 3.0 | 5.2, 14.9 | <0.05 |
| 13 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 14 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 15 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 16 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 17 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 18 | 100 ± 0.0 | 100, 100 | 71 ± 10 | 60.0, 89.6 | <0.05 |
| 19 | 100 ± 0.0 | 100, 100 | 48 ± 15 | 18.0, 69.0 | <0.05 |
| 20 | 100 ± 0.0 | 100, 100 | 51 ± 12 | 37.8, 73.0 | <0.05 |
| 21 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 22 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |

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|----|---------------|----------|---|------|-------|
| 23 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 24 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 25 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 26 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 27 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 28 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 29 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 30 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 31 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 32 | 23 ± 11.0 | 15, 38.0 | 0 | 0, 0 | <0.05 |
| 33 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 34 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 35 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 36 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |

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|----|---------------|----------|--------------|------------|-------|
| 37 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 38 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 39 | 100 ± 0.0 | 100, 100 | 69 ± 5.0 | 58.0, 76.3 | <0.05 |
| 40 | 100 ± 0.0 | 100, 100 | 61 ± 13 | 34.2, 77.4 | <0.05 |
| 41 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 42 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 43 | 100 ± 0.0 | 100, 100 | 84 ± 11 | 61.5, 96.6 | <0.05 |
| 44 | 100 ± 0.0 | 100, 100 | 88 ± 3.0 | 82.3, 94.3 | <0.05 |
| 45 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 46 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 47 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 48 | 100 ± 0.0 | 100, 100 | 72 ± 15 | 46.5, 97.6 | <0.05 |
| 49 | 100 ± 0.0 | 100, 100 | 86 ± 4.7 | 76.5, 91.9 | <0.05 |
| 50 | 100 ± 0.0 | 100, 100 | 75 ± 8.0 | 58.2, 87.1 | <0.05 |

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|----|---------------|----------|--------------|------------|-------|
| 51 | 100 ± 0.0 | 100, 100 | 66 ± 21 | 27.0, 99.6 | <0.05 |
| 52 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 53 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 54 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 55 | 100 ± 0.0 | 100, 100 | 80 ± 7.0 | 72.7, 92.8 | <0.05 |
| 56 | 100 ± 0.0 | 100, 100 | 86 ± 8.0 | 72.7, 98.4 | <0.05 |
| 57 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 58 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 59 | 100 ± 0.0 | 100, 100 | 21 ± 9.0 | 10.3, 39.0 | <0.05 |
| 60 | 100 ± 0.0 | 100, 100 | 78 ± 7.0 | 70.6, 91.8 | <0.05 |
| 61 | 15 ± 6.0 | 3.0, 26 | 0 | 0, 0 | <0.05 |
| 62 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 63 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 64 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |

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|----|-----------------|------------|---|------|-------|
| 65 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 66 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 67 | 27 ± 8.0 | 12.0, 38.0 | 0 | 0, 0 | <0.05 |
| 68 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 69 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 70 | 45.2 ± 19.0 | 23.5, 8 | 0 | 0, 0 | <0.05 |
| 71 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 72 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 73 | 35 ± 14 | 19.0, 61.0 | 0 | 0, 0 | <0.05 |
| 74 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 75 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 76 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 77 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 78 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |

| | | | | | |
|----|----------------|------------|-----------------|------------|-------|
| 79 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 80 | 100 ± 0.0 | 100, 100 | 51 ± 15 | 24.5, 75.4 | <0.05 |
| 81 | 100 ± 0.0 | 100, 100 | 73 ± 10 | 42.6, 98.1 | <0.05 |
| 82 | 17 ± 12 | 5.2, 40.7 | 0 | 0, 0 | <0.05 |
| 83 | 52 ± 11 | 38.6, 64.6 | 0 | 0, 0 | <0.05 |
| 84 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 85 | 100 ± 0.0 | 100, 100 | 72 ± 5 | 66.8, 81.0 | <0.05 |
| 86 | 100 ± 0.0 | 100, 100 | 85 ± 7 | 71.4, 94.7 | <0.05 |
| 87 | 100 ± 0.0 | 100, 100 | 81 ± 3 | 78.0, 87.7 | <0.05 |
| 88 | 9 ± 2 | 6, 13 | 0 | 0, 0 | <0.05 |
| 89 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 90 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 91 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 92 | 87.4 ± 3.7 | 83.2, 94.2 | 27.2 ± 16.6 | 8.9, 59.7 | <0.05 |

| | | | | | |
|-----|----------------|------------|---------------|----------|-------|
| 93 | 47.9 ± 8.3 | 38.5, 63.2 | 0 | 0, 0 | <0.05 |
| 94 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 95 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 96 | 53 ± 18 | 32.1, 86.2 | 0 | 0, 0 | <0.05 |
| 97 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 98 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 99 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 100 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 101 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 102 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 103 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 104 | 16 ± 3.0 | 13.0, 19.0 | 0 | 0, 0 | <0.05 |
| 105 | 59 ± 2.1 | 56.5, 62.8 | 100 ± 0.0 | 100, 100 | <0.05 |
| 106 | 32.5 ± 5.0 | 36.5, 38.5 | 100 ± 0.0 | 100, 100 | <0.05 |

| | | | | | |
|-----|----------------|------------|---------------|------------|-------|
| 107 | 22.6 ± 9.4 | 4.7, 36.8 | 0 | 0, 0 | <0.05 |
| 108 | 100 ± 0.0 | 100, 100 | 51 ± 13 | 37.5, 76.1 | <0.05 |
| 109 | 69.6 ± 5.4 | 63.3, 79.5 | 100 ± 0.0 | 100, 100 | <0.05 |
| 110 | 100 ± 0.0 | 100, 100 | 88 ± 14 | 47.1, 64.8 | <0.05 |
| 111 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 112 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 113 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 114 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 115 | 88 ± 3.9 | 81.4, 94.9 | 5.5 ± 5.3 | 0.1,16.0 | <0.05 |
| 116 | 90 ± 3 | 84, 95 | 0 | 0, 0 | <0.05 |
| 117 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 118 | 35 ± 14 | 30, 39 | 0 | 0, 0 | <0.05 |
| 119 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 120 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |

| | | | | | |
|-----|---------------|----------|---|------|-------|
| 121 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 122 | 100 ± 0.0 | 100, 100 | 0 | 0, 0 | <0.05 |
| 1' | N/A | -- | | | <0.05 |
| 2' | N/A | -- | | | <0.05 |
| 3' | N/A | -- | | | <0.05 |
| 4' | N/A | -- | | | <0.05 |
| 5' | N/A | -- | | | <0.05 |
| 6' | N/A | -- | | | <0.05 |
| 7' | N/A | -- | | | <0.05 |
| 8' | N/A | -- | | | <0.05 |
| 9' | N/A | -- | | | <0.05 |
| 10' | N/A | -- | | | <0.05 |
| 11' | N/A | -- | | | <0.05 |

¹Gel position of marked spots is shown in Fig. 2.

²Mean values and standard error of *PR*.

³Non-parametric bootstrap CIs (CL, lower bound; CU, upper bound) determined by the bias corrected percentile method.

⁴Statistically significant differences ($P < 0.05$) in phosphorylation rate between methods.

N/A: More volume in untreated than treated spot with HF-P method

Table S2

Phosphorylation rate (*PR*) of each replicate and mean (\pm SE) for 2-DE spots with statistically significant different *PR* between two methods (HF-P and Pro-Q DPS) to evaluate phosphorylation in meat samples from LT muscle.

| Spot code ¹ | <i>PR</i> _{HF-P} | | | | <i>PR</i> _{Pro-Q DPS} | | | |
|---------------------------|---------------------------|----------------|----------------|----------------------------|--------------------------------|----------------|----------------|----------------------------|
| | Replicate 1 | Replicate 2 | Replicate 3 | Mean \pm SE ² | Replicate 1 | Replicate 2 | Replicate 3 | Mean \pm SE ² |
| 1 | 100 | -- | 100 | 100 \pm 0.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 2 | 100 | 100 | 100 | 100 \pm 0.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 3 | -- | 100 | 100 | 100 \pm 0.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 4 | 100 | 100 | 100 | 100 \pm 0.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 5 | 69.9 | 33.3 | -- | 52.0 \pm 15.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 6 | 51.9 | 22.5 | 66.8 | 47 \pm 13.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 7 | 100 | 100 | 100 | 100 \pm 0.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 8 | 100 | 100 | 100 | 100 \pm 0.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |
| 9 | 100 | 100 | 100 | 100 \pm 0.0 | 0 | 0 | 0 | 0.0 \pm 0.0 |

| | | | | | | | | |
|----|-----|-----|-----|---------------|-------|-------|-------|---------------|
| 10 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 11 | 100 | 100 | 100 | 100 ± 0.0 | 14.56 | 7.02 | 18.38 | 13 ± 3.0 |
| 12 | 100 | 100 | 100 | 100 ± 0.0 | 14.93 | 5.21 | 10.59 | 10 ± 3.0 |
| 13 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 14 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 15 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 16 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 17 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 18 | 100 | 100 | 100 | 100 ± 0.0 | 57.44 | 65.11 | 89.63 | 71 ± 10 |
| 19 | 100 | 100 | 100 | 100 ± 0.0 | 17.98 | 69.04 | 57.49 | 48 ± 15 |
| 20 | 100 | 100 | 100 | 100 ± 0.0 | 47.09 | 33.18 | 73.03 | 51 ± 12 |
| 21 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 22 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 23 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 24 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |

| | | | | | | | | |
|----|------|-----|------|---------------|-------|-------|-------|---------------|
| 25 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 26 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 27 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 28 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 29 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 30 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 31 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 32 | 38.3 | 1.5 | 29.3 | 23 ± 11.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 33 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 34 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 35 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 36 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 37 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 38 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 39 | 100 | 100 | 100 | 100 ± 0.0 | 76.29 | 58.03 | 72.04 | 69 ± 5.0 |

| | | | | | | | | |
|----|-----|-----|-----|---------------|-------|-------|-------|---------------|
| 40 | 100 | 100 | 100 | 100 ± 0.0 | 34.19 | 77.43 | 70.54 | 61 ± 13 |
| 41 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 42 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 43 | 100 | 100 | 100 | 100 ± 0.0 | 96.63 | 61.54 | 91.41 | 84 ± 11 |
| 44 | 100 | 100 | 100 | 100 ± 0.0 | 94.33 | 82.33 | 89.10 | 88 ± 3.0 |
| 45 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 46 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 47 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 48 | 100 | 100 | 100 | 100 ± 0.0 | 46.52 | 73.14 | 97.58 | 72 ± 15 |
| 49 | 100 | 100 | 100 | 100 ± 0.0 | 89.07 | 76.55 | 91.90 | 86 ± 5.0 |
| 50 | 100 | 100 | 100 | 100 ± 0.0 | 87.13 | 80.49 | 58.17 | 75 ± 8.0 |
| 51 | 100 | 100 | 100 | 100 ± 0.0 | 99.65 | 26.97 | 71.92 | 66 ± 21 |
| 52 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 53 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 54 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |

| | | | | | | | | |
|----|------|------|------|---------------|-------|-------|-------|---------------|
| 55 | 100 | 100 | 100 | 100 ± 0.0 | 92.80 | 75.21 | 71.59 | 80 ± 7.0 |
| 56 | 100 | 100 | 100 | 100 ± 0.0 | 89.0 | 72.0 | 98.0 | 86 ± 8.0 |
| 57 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 58 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 59 | 100 | 100 | 100 | 100 ± 0.0 | 7.36 | 39.2 | 16.18 | 21 ± 9.0 |
| 60 | 100 | 100 | 100 | 100 ± 0.0 | 91.83 | 71.18 | 70.29 | 78 ± 7.0 |
| 61 | 26.2 | 3.4 | 15.2 | 15 ± 6.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 62 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 63 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 64 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 65 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 66 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 67 | 12.2 | 31.7 | 38.0 | 27 ± 8.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 68 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 69 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |

| | | | | | | | | |
|----|------|------|------|-----------------|-------|-------|-------|---------------|
| 70 | 14.4 | 41.6 | 79.8 | 45.2 ± 19.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 71 | 100 | 100 | -- | 100 ± 0.0 | 0 | 0 | -- | 0.0 ± 0.0 |
| 72 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 73 | 12.0 | 33.5 | 60.7 | 35 ± 14 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 74 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 75 | 100 | 100 | -- | 100 ± 0.0 | 0 | 0 | -- | 0.0 ± 0.0 |
| 76 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 77 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 78 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 79 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 80 | 100 | 100 | 100 | 100 ± 0.0 | 24.56 | 53.10 | 75.39 | 51 ± 15 |
| 81 | 100 | 100 | -- | 100 ± 0.0 | 42.59 | 79.20 | 98.13 | 73 ± 16 |
| 82 | 5.2 | 5.2 | 40.7 | 17 ± 12 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 83 | 38.6 | -- | 64.7 | 52 ± 11 | 0 | -- | 0 | 0.0 ± 0.0 |
| 84 | 100 | 100 | -- | 100 ± 0.0 | 0 | 0 | -- | 0.0 ± 0.0 |

| | | | | | | | | |
|----|------|------|------|----------------|-------|-------|-------|-----------------|
| 85 | 100 | 100 | 100 | 100 ± 0.0 | 69.89 | 65.28 | 80.98 | 72 ± 5 |
| 86 | 100 | 100 | 100 | 100 ± 0.0 | 71.38 | 90.06 | 94.67 | 85 ± 7 |
| 87 | 100 | 100 | 100 | 100 ± 0.0 | 77.02 | 87.67 | 79.97 | 81 ± 3 |
| 88 | 13.4 | 7.6 | 5.3 | 9 ± 2 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 89 | 100 | -- | 100 | 100 ± 0.0 | 0 | -- | 0 | 0.0 ± 0.0 |
| 90 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 91 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 92 | 81.5 | 94.2 | 86.6 | 87.4 ± 3.7 | 4.99 | 16.85 | 59.74 | 27.2 ± 16.6 |
| 93 | 34.8 | 45.8 | 63.3 | 47.9 ± 8.3 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 94 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 95 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 96 | 24.1 | 48.0 | 86.2 | 53 ± 18 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 97 | 100 | -- | 100 | 100 ± 0.0 | 0 | -- | 0 | 0.0 ± 0.0 |
| 98 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 99 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |

| | | | | | | | | |
|-----|------|------|------|----------------|-------|-------|-------|---------------|
| 100 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 101 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 102 | -- | 100 | 100 | 100 ± 0.0 | -- | 0 | 0 | 0.0 ± 0.0 |
| 103 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 104 | -- | 19.4 | 13.0 | 16 ± 3.0 | -- | 0 | 0 | 0.0 ± 0.0 |
| 105 | 63.0 | 58.8 | 55.5 | 59 ± 2.1 | 100 | 100 | 100 | 100 ± 0.0 |
| 106 | 38.5 | 22.5 | 36.5 | 32.5 ± 5.0 | 100 | 100 | 100 | 100 ± 0.0 |
| 107 | 4.7 | 36.8 | 26.6 | 22.6 ± 9.4 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 108 | 100 | 100 | 100 | 100 ± 0.0 | 34.93 | 76.15 | 42.67 | 51 ± 13 |
| 109 | 60.8 | 68.5 | 79.5 | 69.6 ± 5.4 | 100 | 100 | 100 | 100 ± 0.0 |
| 110 | 100 | -- | 100 | 100 ± 0.0 | 100 | -- | 70.38 | 88 ± 14 |
| 111 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 112 | -- | 100 | 100 | 100 ± 0.0 | -- | 0 | 0 | 0.0 ± 0.0 |
| 113 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 114 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |

| | | | | | | | | |
|-----|-------|-------|-------|---------------|----|-------|------|---------------|
| 115 | 94.91 | 81.38 | 90.06 | 88 ± 3.9 | 0 | 16.03 | 0.42 | 5.5 ± 5.3 |
| 116 | 94.9 | 83.9 | 90.0 | 90 ± 3 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 117 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 118 | -- | 30.0 | 39.2 | 35 ± 14 | -- | 0 | 0 | 0.0 ± 0.0 |
| 119 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 120 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 121 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 122 | 100 | 100 | 100 | 100 ± 0.0 | 0 | 0 | 0 | 0.0 ± 0.0 |
| 1' | N/A | N/A | N/A | | | | | |
| 2' | N/A | N/A | N/A | | | | | |
| 3' | N/A | N/A | N/A | | | | | |
| 4' | N/A | N/A | N/A | | | | | |
| 5' | N/A | N/A | N/A | | | | | |
| 6' | N/A | N/A | N/A | | | | | |
| 7' | N/A | N/A | N/A | | | | | |

| | | | |
|-----|-----|-----|-----|
| 8' | N/A | N/A | N/A |
| 9' | N/A | N/A | N/A |
| 10' | N/A | N/A | N/A |
| 11' | N/A | N/A | N/A |

¹ Gel positions of marked spots is shown in Figure 2

²Mean values and standard error of *PR*.

N/A: More volume in untreated than treated spot with HF-P method

Table S3

Volume of spots for untreated protein samples, dephosphorylated spots with HF-P and phosphorylated spots visualized with Pro-Q DPS were assessed by PDQuest software.

| Spot code ¹ | Volume/Total protein ² | | | | Volume/ HF-P treatment ² | | | | Volume/ Pro-Q DPS ² | | | |
|---------------------------|-----------------------------------|--------|--------|-----------------|-------------------------------------|--------|--------|-----------------|--------------------------------|--------|--------|-----------------|
| | Repl 1 | Repl 2 | Repl 3 | Mean ± | Repl 1 | Repl 2 | Repl 3 | Mean ± | Repl 1 | Repl 2 | Repl 3 | Mean ± |
| | | | | SE ³ | | | | SE ³ | | | | SE ³ |
| 1A | 3561 | 1387 | 2737 | 2562 ± 634 | 0 | 0 | 0 | 0 ± 0 | 0 | 0 | 0 | 0 ± 0 |
| 2A | 12396 | 7724.2 | 19575 | 13232 ± 3446 | 2295 | 446 | 2616 | 1786 ± 676 | 619 | 1301 | 11694 | 4538 ± 3583 |
| 3A | 9611 | 14224 | 18967 | 14267 ± 2701 | 6266 | 7706 | 6966 | 6979 ± 416 | 0 | 0 | 0 | 0 ± 0 |
| 4A | 1136 | 8001 | -- | 4568 ± 2803 | 0 | 0 | 0 | 0 ± 0 | 0 | 0 | 0 | 0 ± 0 |
| 5A | 2130 | 2393 | 1451 | 1991 ± 281 | 5584 | 7758 | 4382 | 5908 ± 988 | 0 | 0 | 0 | 0 ± 0 |

| | | | | | | | | | | | | |
|----|-------|-------|-------|-----------------|------|------|------|------------|-------|-------|-------|-----------------|
| 1B | 6284 | 3904 | 5587 | 5258 ± 706 | 2337 | 1607 | 2487 | 2144 ± 272 | 14700 | 9875 | 10748 | 11774 ± 1484 |
| 2B | 7564 | 5482 | 9379 | 7475 ± 1126 | 4651 | 4247 | 5955 | 4951 ± 515 | 14352 | 9697 | 9554 | 11201 ± 1576 |
| 3B | 2653 | 3816 | 4182 | 3550 ± 461 | 2529 | 2413 | 3070 | 2671 ± 202 | 0 | 0 | 0 | 0 ± 0 |
| 4B | 23123 | 17493 | 10743 | 17120 ± 3579 | 0 | 0 | 0 | 0 ± 0 | 37437 | 48448 | 48497 | 44794 ± 3678 |
| 5B | 18864 | 20171 | 29351 | 22795 ± 3299 | 7401 | 6360 | 6008 | 6590 ± 418 | 63043 | 50724 | 66758 | 60175 ± 4845 |
| 6B | 2089 | 4847 | 18832 | 8589 ± 5183 | 4234 | 3029 | 1905 | 3056 ± 672 | 705 | 4232 | 6496 | 3811 ± 1685 |
| 7B | -- | -- | -- | -- | 3429 | 4671 | 3380 | 3826 ± 422 | -- | -- | -- | -- |
| 1C | 1657 | 1275 | 4330 | 2421 ± 961 | 0 | 0 | 0 | 0 ± 0 | 0 | 0 | 0 | 0 ± 0 |
| 2C | 2962 | 7239 | 7749 | 5983 ± 1518 | 0 | 0 | 0 | 0 ± 0 | 0 | 0 | 0 | 0 ± 0 |

| | | | | | | | | | | | | |
|----|-------|-------|-------|-----------------|-----|------|------|------------|---|------|----|--------------|
| 3C | 12496 | 15407 | 11677 | 13193 ± 1132 | 636 | 2868 | 1161 | 1555 ± 674 | 0 | 2469 | 49 | 839 ± 814 |
|----|-------|-------|-------|-----------------|-----|------|------|------------|---|------|----|--------------|

¹ Gel positions of marked spots is shown in Figure 4.

² Volume of each biological replicate.

³ Mean (± SE) volumes from replicates of *PR*

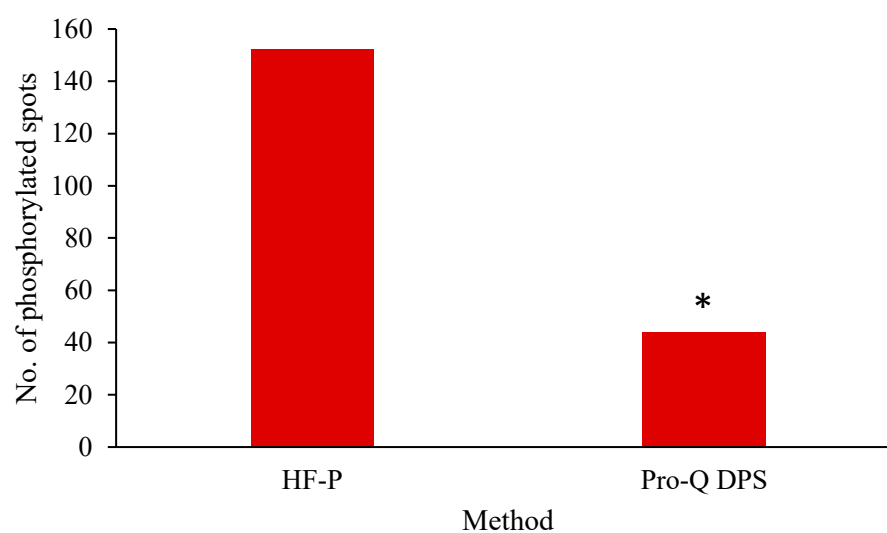


Figure S1. Number of phosphorylated protein spots obtained with HF-P and Pro-Q DPS method (* $P < 0.0001$, Fisher's exact test).