Supporting Information

Phosphoinositide binding by the PH domain in ceramide transfer protein (CERT) is inhibited by hyperphosphorylation of an adjacent serine-repeat motif

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Running title: *Functional regulation of CERT PH by phosphorylation of SRM*

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Supporting Figures

Fig. S1

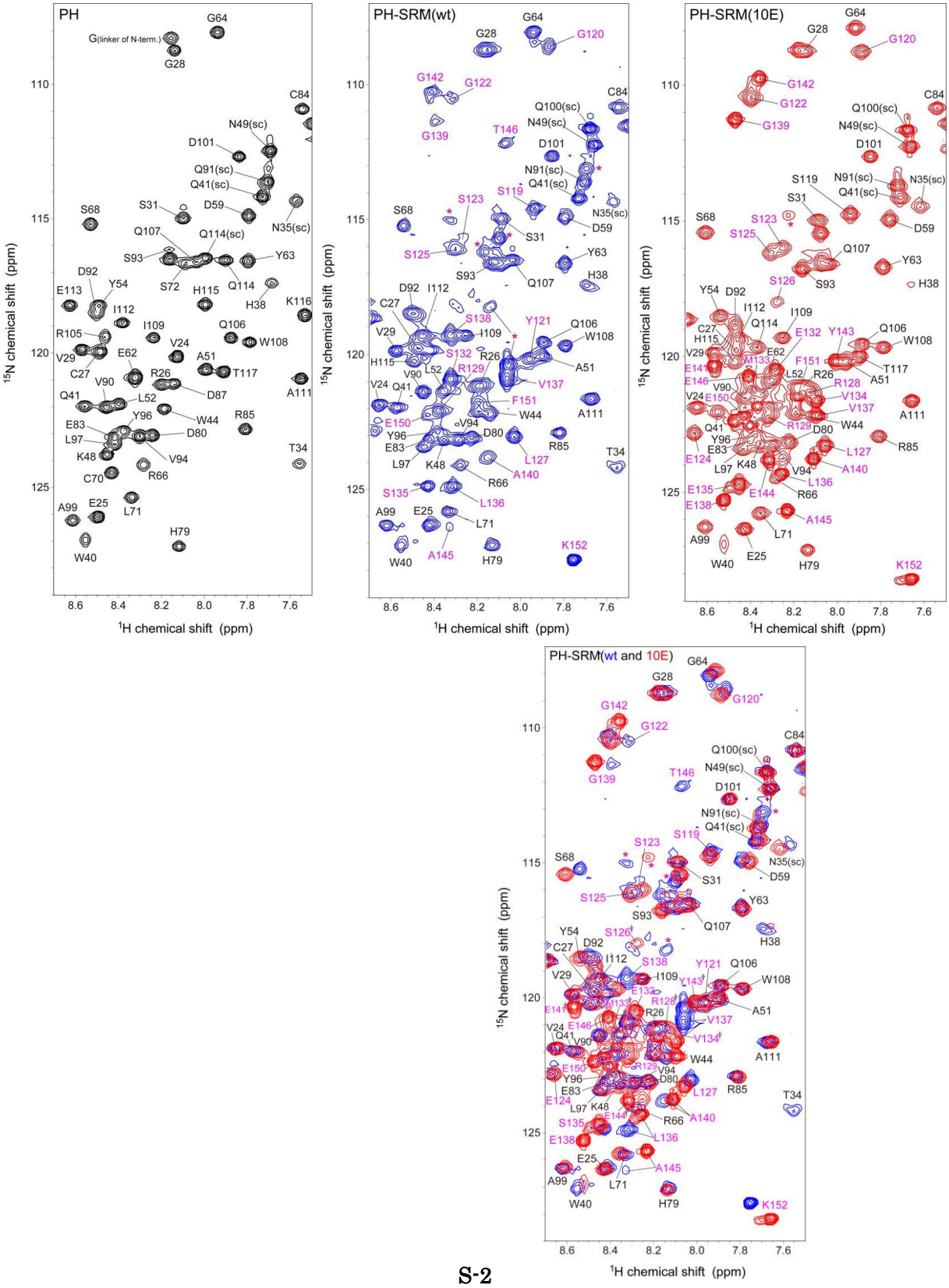

FIGURE S1. Effects of the 10E mutation on correlation signals derived from the non-PH region (corresponding to amino acids 118–152 in PH-SRM). The region of 2D ^{1}H-^{15}N HSQC spectra specified by the gray dotted lined box in Fig. 1A are enlarged (upper panels), and the superimposed spectra of PH-SRM(wt) and its 10E form are shown (lower panel). The correlation signals derived from the non-PH region (assignments of signals are denoted in magenta) are faintly visible in the narrow chemical shift regions shown. The signals, which were visible only in PH-SRM(10E), were indicated by obelisks, as superscript of assignment labels on the superimposed NMR spectra. Those signals with stars in the spectra are unassigned due to severe line broadening of 3D NMR spectra.

Fig. S2

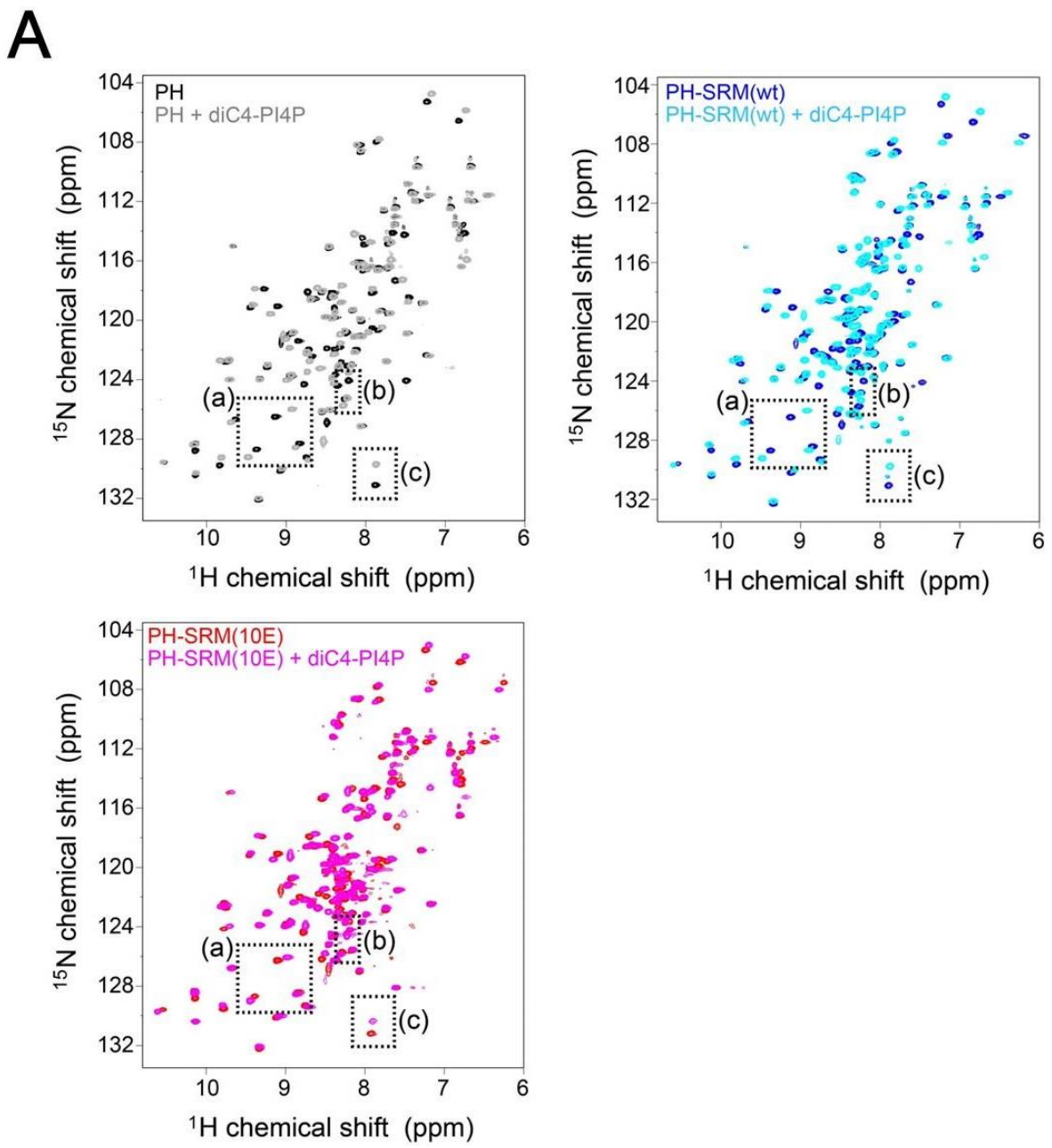
A
PH
PH + diC4-PI4P
PH-SRM(wt)
PH-SRM(wt) + diC4-PI4P
PH-SRM(10E)
PH-SRM(10E) + diC4-PI4P
(a)
(b)
(c)
^{15}N chemical shift (ppm)
^{1}H chemical shift (ppm)
104
108
112
116
120
124
128
132
10
9
8
7
6

Fig. S2 (continued)

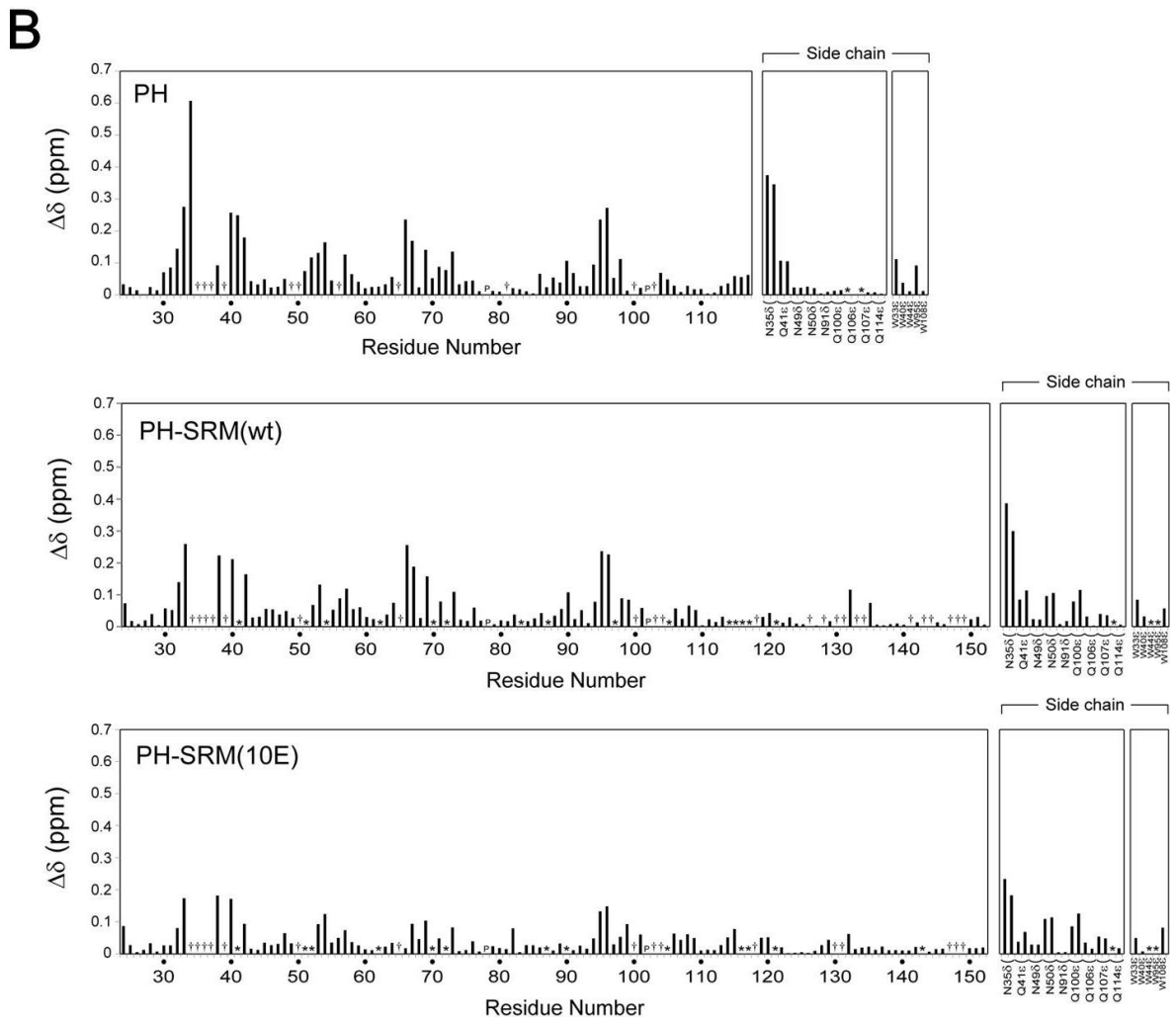

FIGURE S2. The hyperphosphorylation-mimetic SRM attenuates the degree of chemical shift changes in the CERT PH domain upon the addition of diC$_4$-PI4P. *A*, The superposition of the 2D ^{1}H-^{15}N HSQC spectra of CERT PH (left panel), PH-SRM(wt) (center panel), and PH-SRM(10E) (right panel) is shown. *B*, Normalized chemical shift differences (Δδ) in individual residues of CERT PH (upper panel), PH-SRM(wt) (middle panel), and PH-SRM(10E) (lower panel) upon interaction with diC$_4$-PI4P. As described in the caption of Fig. 2 and the section on Experimental Procedures, the protein concentration of those NMR samples was 0.1 m*M* and the Δδ values of each amino acid residue in the presence or absence of 1.6 m*M* diC$_4$-PI4P were analyzed. The signals, which disappeared or were severely degenerated, were indicated by obelisks or asterisks, respectively. The "P" indicates proline

residues (Pro78 and Pro102).

Fig. S3

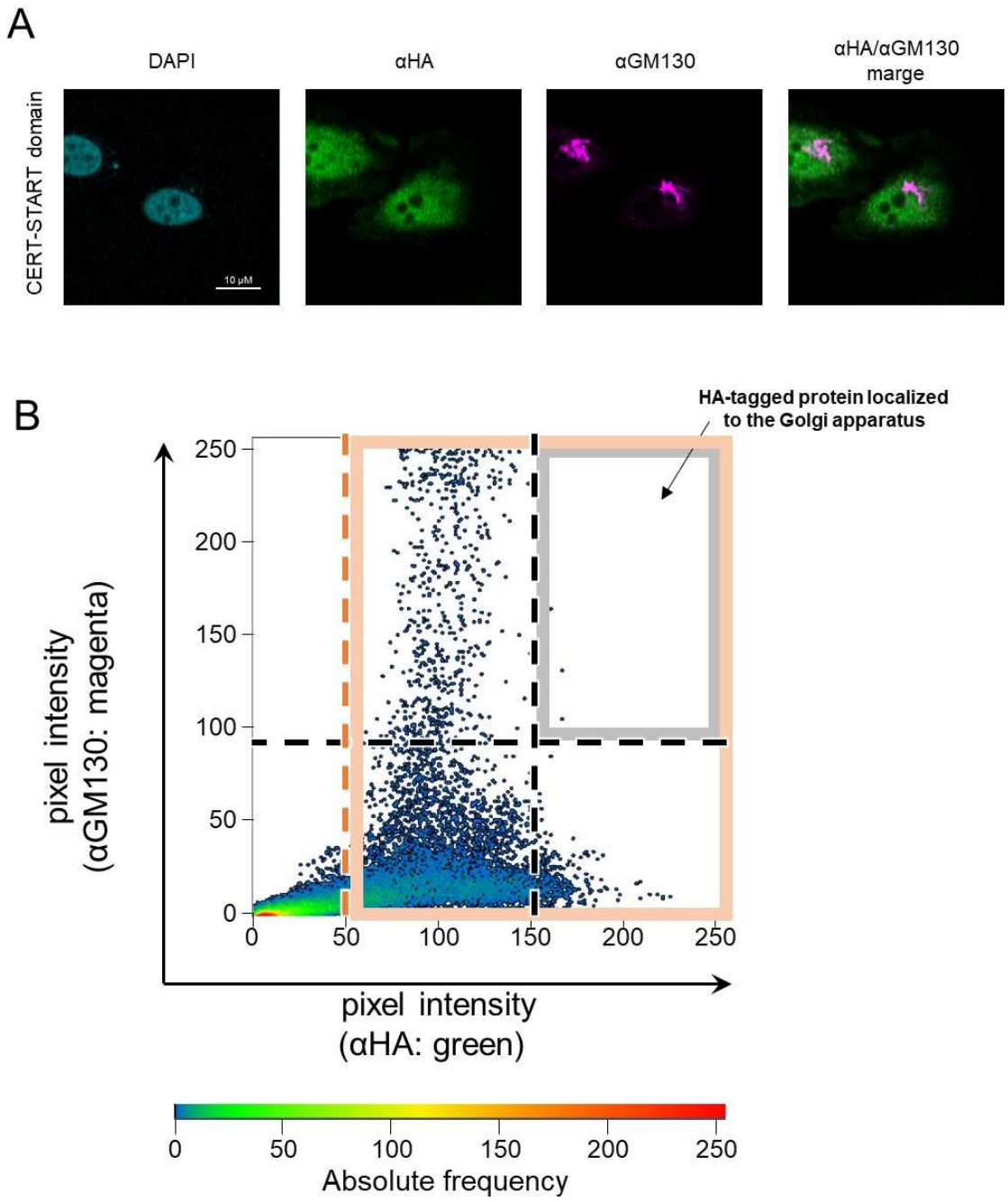

FIGURE S3. Selection of a pixel area co-localized with the Golgi marker GM130. The HA-CERT START domain, which has no ability to associate specifically with the Golgi apparatus, was used as a negative control to set the pixel area colocalized with GM130. *A*, HeLa cells expressing the HA-CERT START domain were double-labeled with the anti-HA antibody (green) and anti-GM130 antibody (magenta) by indirect immunostaining and were

observed by confocal microscopy. Typical staining patterns are shown. The bar indicates 10 μm. *B*, A pixel intensity 2D map of the anti-HA signal (horizontal) and anti-GM130 signal (vertical) in HeLa cells expressing the HA-tagged START domain shown in panel *A* was drawn using ZEN software (Carl Zeiss). In order to select the co-localized pixel area (the upper right area), cross-hair bars were set to represent the border between Golgi and non-Golgi areas in the 2D map (the x-axis; to exclude Golgi areas in the background level, the y-axis; to exclude nuclear and cytoplasm areas).

Fig. S4

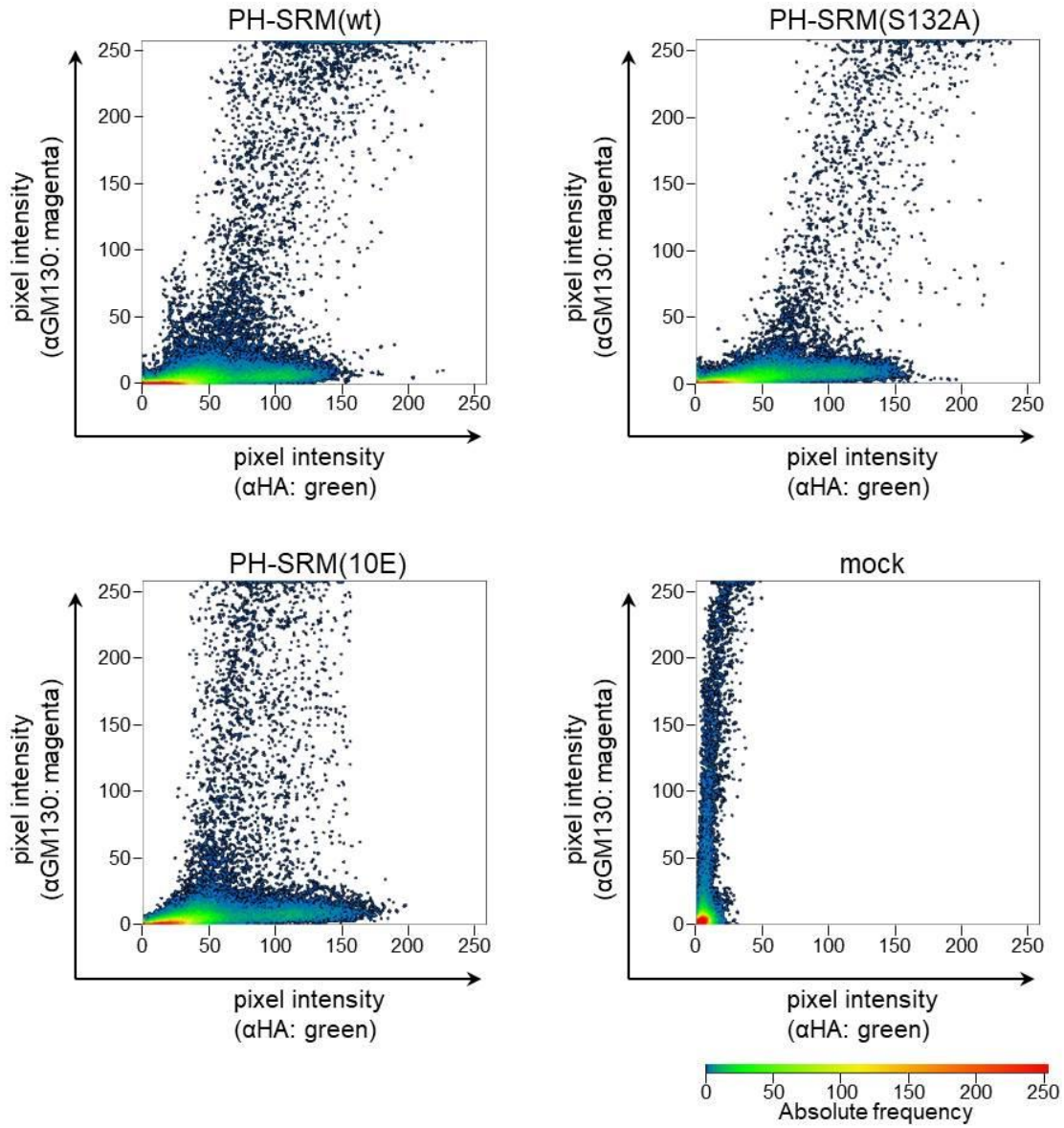

FIGURE S4. Pixel intensity 2D map of the immunostaining pattern shown in Fig. 4B. A pixel intensity 2D map of the anti-HA signal (horizontal) and anti-GM130 signal (vertical) in HeLa cells shown in Fig. 4B was drawn using ZEN software (Carl Zeiss).