# Supplemental Information

# CF-PA²Vtech: a cell-free human protein array technology for antibody validation against human proteins

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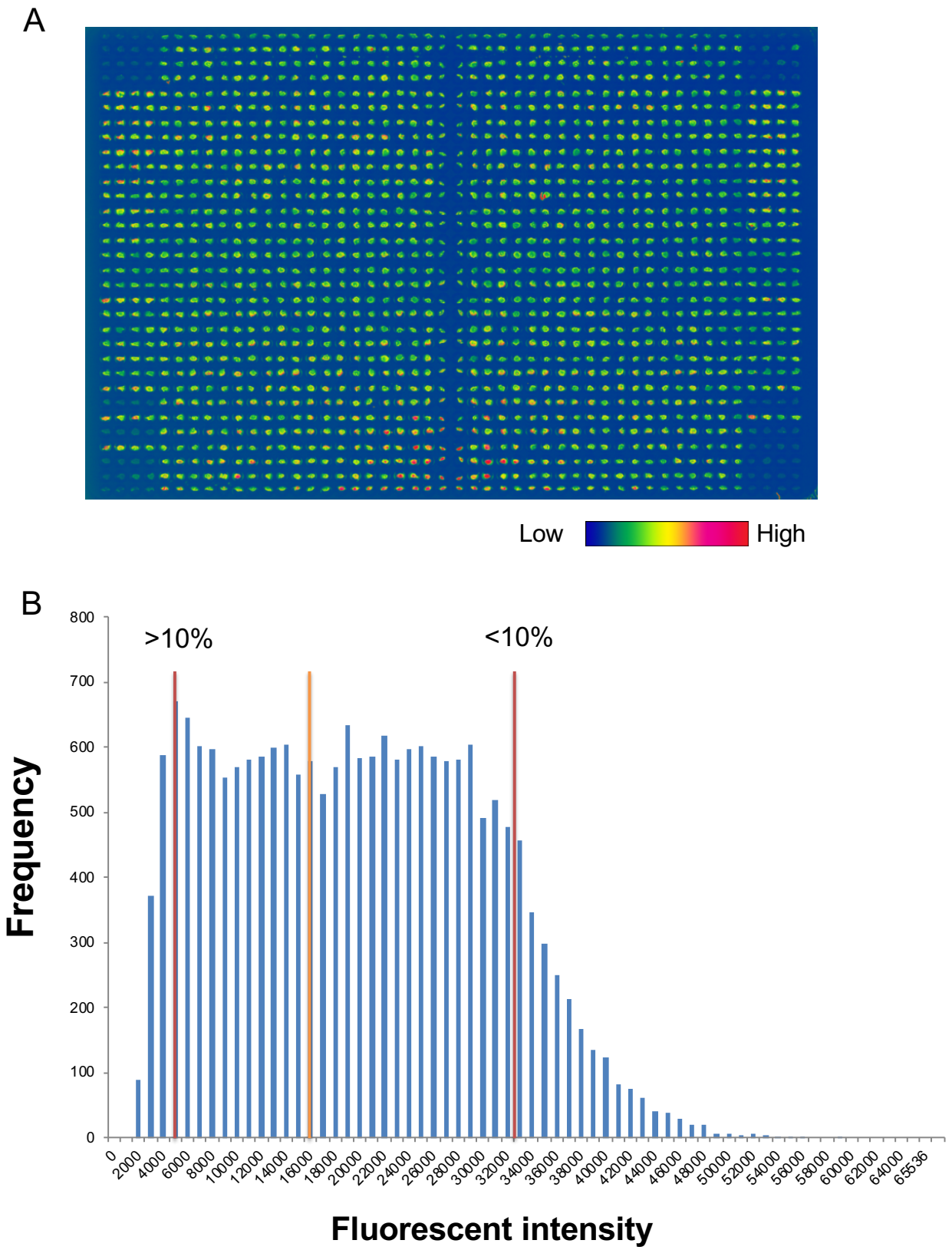
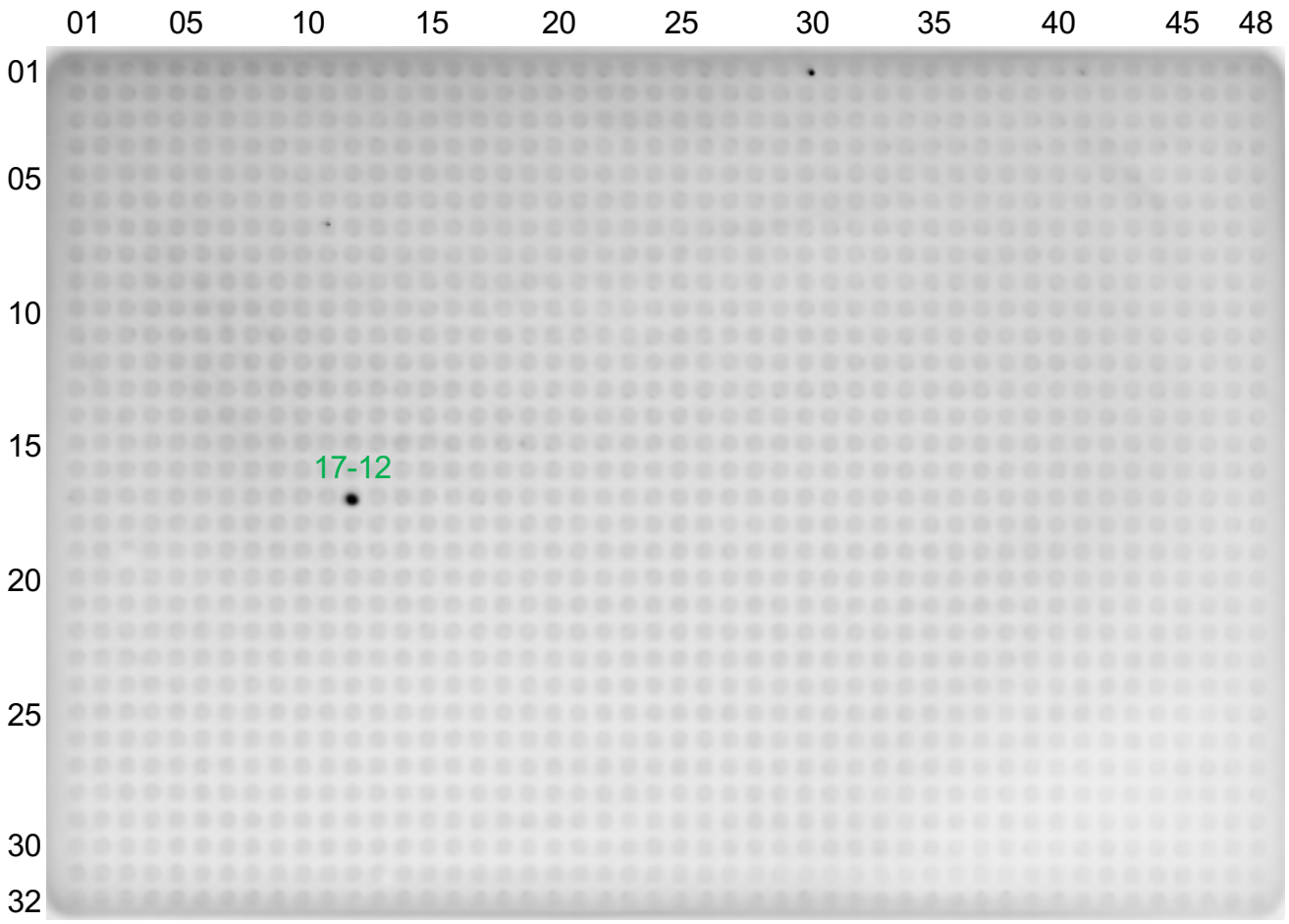
# Supplementary Figures 1-6



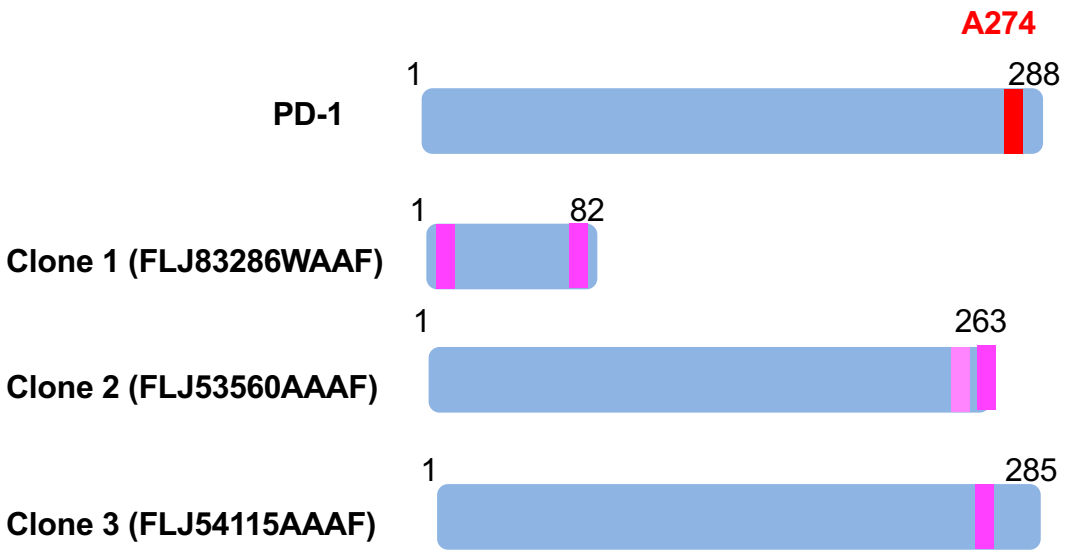

**Supplementary Figure 1. Frequency of fluorescent intensity on 1536-well format.**

Arrayed proteins were detected using a fluorescence-labeled anti-FLAG antibody, and the diversity of fluorescence intensity of each arrayed protein was investigated. Typhoon FLA 9500 (GE Healthcare UK Ltd.) was used for fluorescence detection. Array-Pro Analyzer was used for signal quantification. Fluorescence intensity showed that more than 80% of wells were found within the high signal zone.



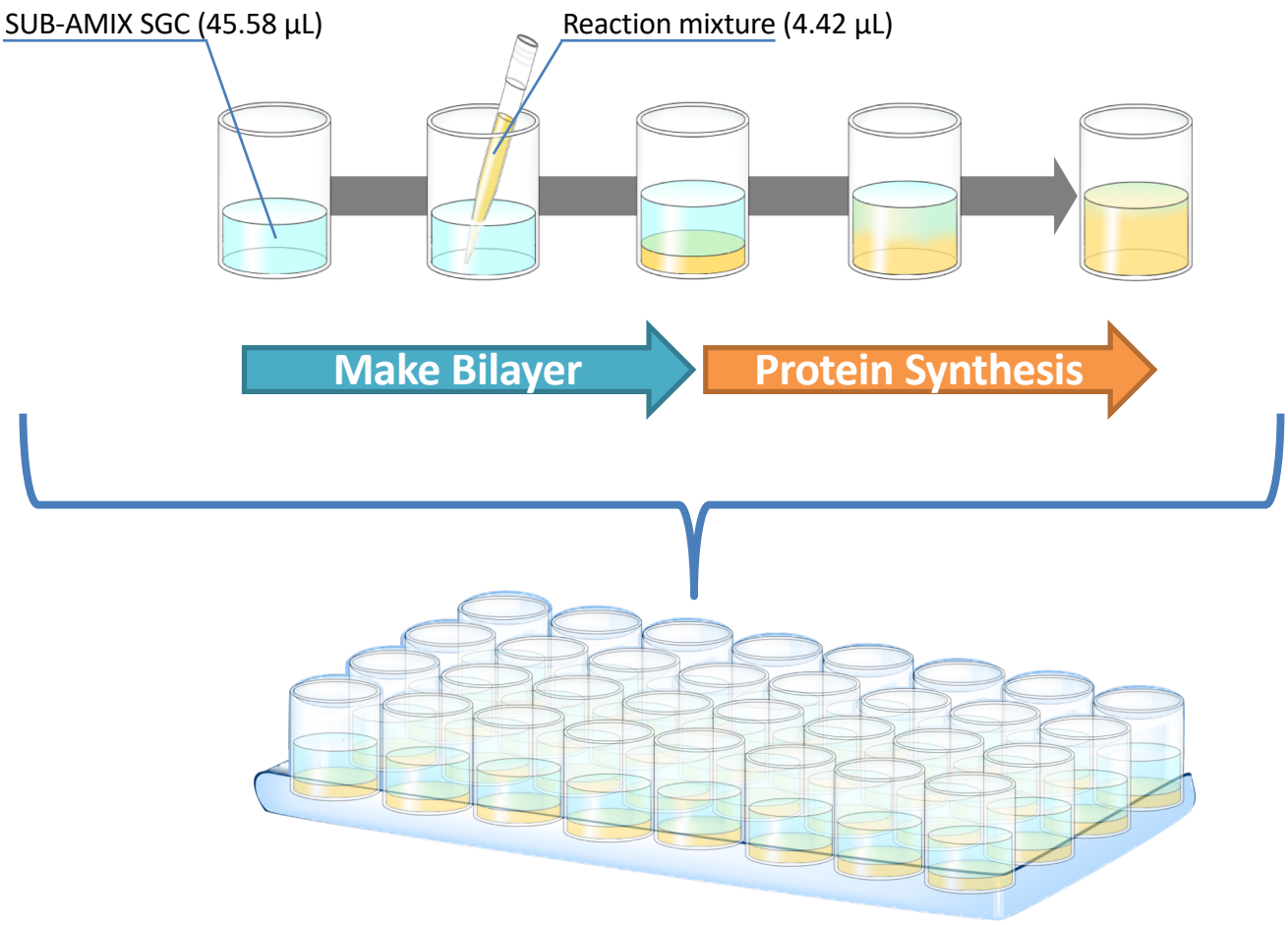

**Supplementary Figure 2. Results of reaction by anti-rabbit IgG antibody on a single 1536-well format.**

Single spot (17-12) showed cross-reactivity against anti-rabbit IgG antibody.




**Supplementary Figure 3. Location of similar epitope sequences in cross-reactive proteins using anti-PD-1 antibody.**

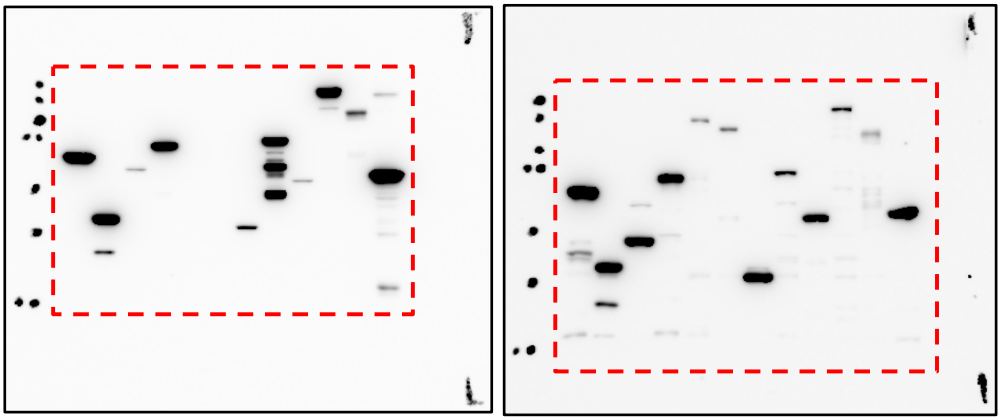
Clone 1 (FLJ83286WAAF) and Clone 2 (FLJ53560AAAF), or Clone 3 (FLJ54115AAAF) have two or one different candidate epitope sequences, respectively. The candidate epitope is shown as the pink colour bar. Red colour bar in PD-1 protein denotes a position of antigen peptide of anti-PD-1 antibody used.




**Supplementary Figure 4. Protein synthesis scheme using a wheat cell-free protein synthesis on 384-well plate.**

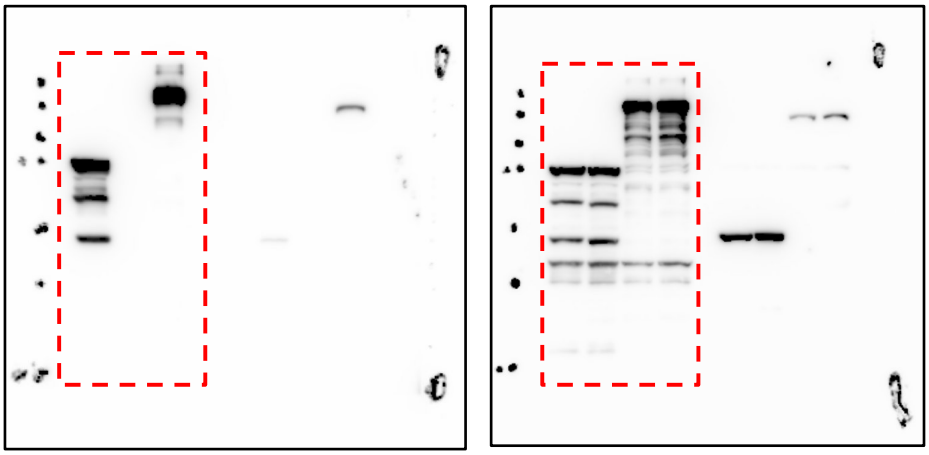
A protein was synthesized in a well on 384-well plate. All dispensing processes for protein synthesis were carried out by a fully automatic dispenser HTS10-HD.

Figure 3A



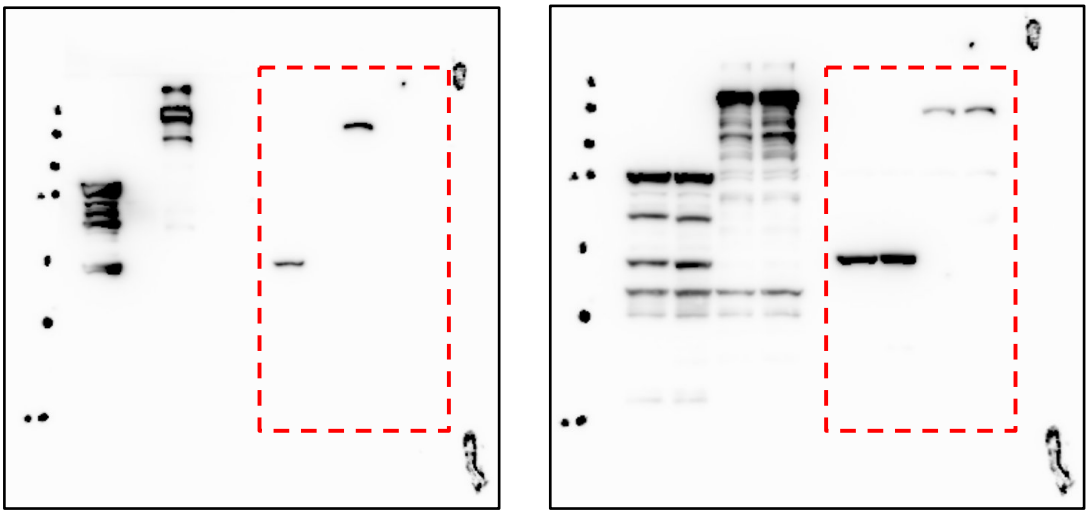
IB: HA IB: FLAG

Figure 3B



IB: HA IB: FLAG

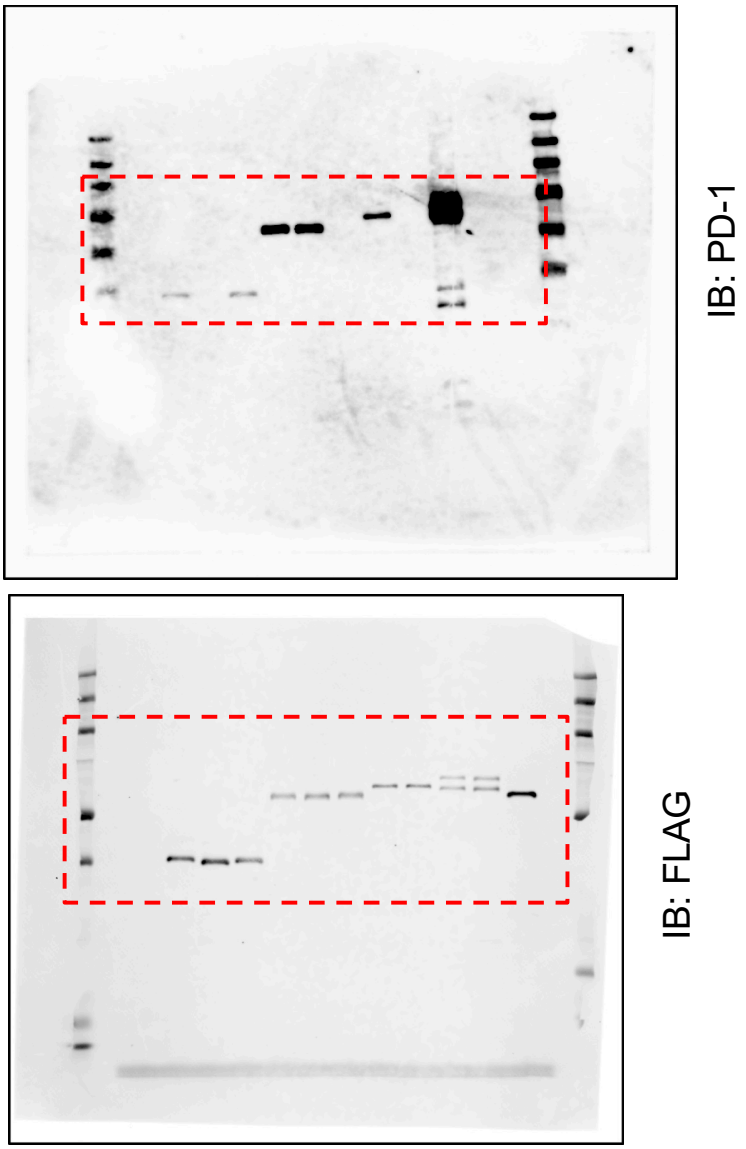
Figure 3C



IB: HA IB: FLAG

**Supplementary Figure 5. Uncropped immunoblots in Figure 3.**

Figure 5B




**Supplementary Figure 6. Uncropped immunoblots in Figure 5.**