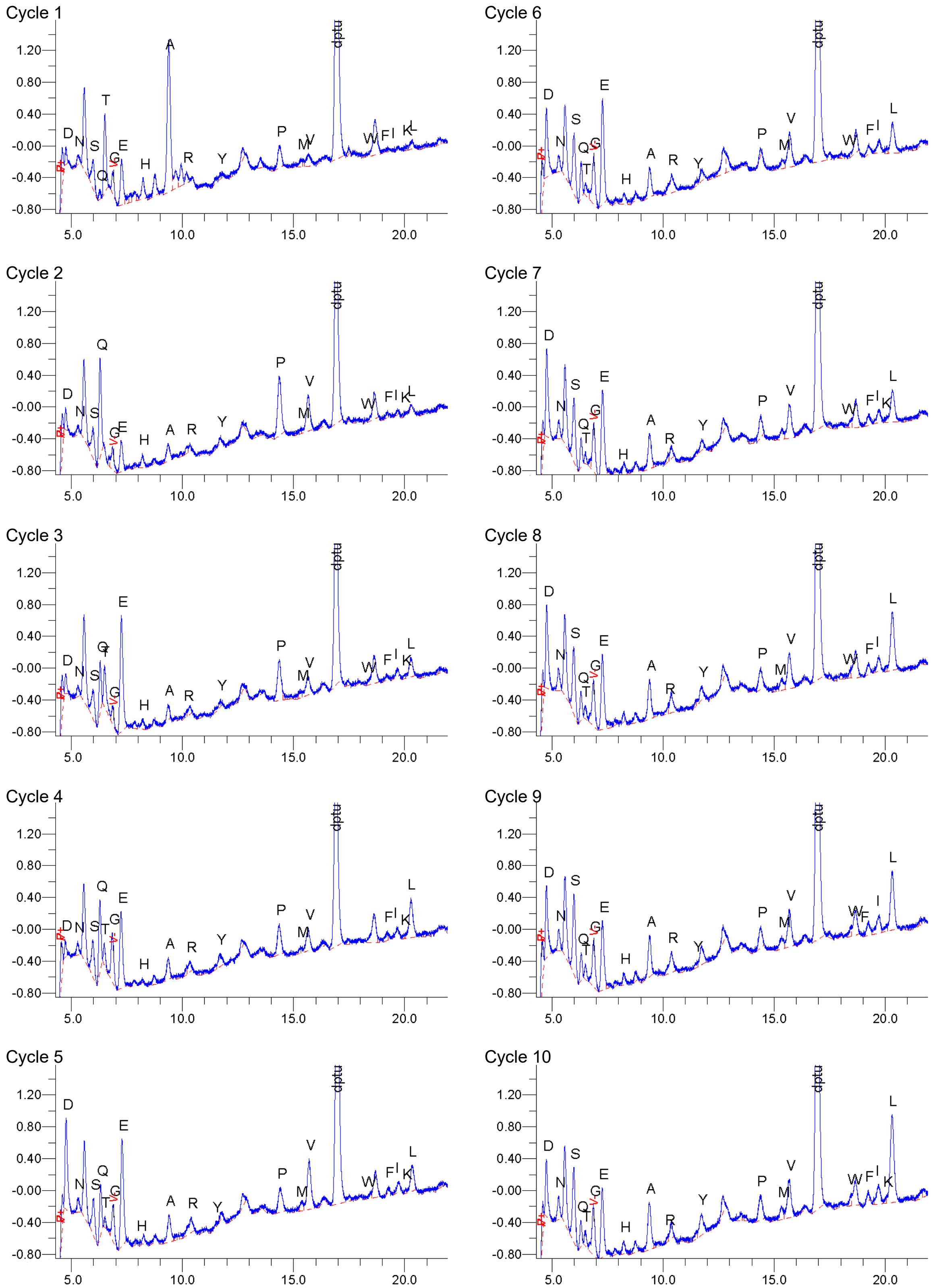

Fig. S1. N-terminal sequence analysis of Fragment 2. Ms Jaw1 NIDR PA (Fig. 3F) was expressed in HEK293 cells and immunoprecipitated with an anti-PA antibody. After SDS-PAGE and electrotransfer to a PVDF membrane, the protein band corresponding to Fragment 2 (Fig. 3H) was applied on a protein sequencer, on which Edman microsequencing was performed for 10 cycles. Resultant phenylthiohydantoin-amino acids (PTH-AA) were separated automatically on a C18 HPLC column.

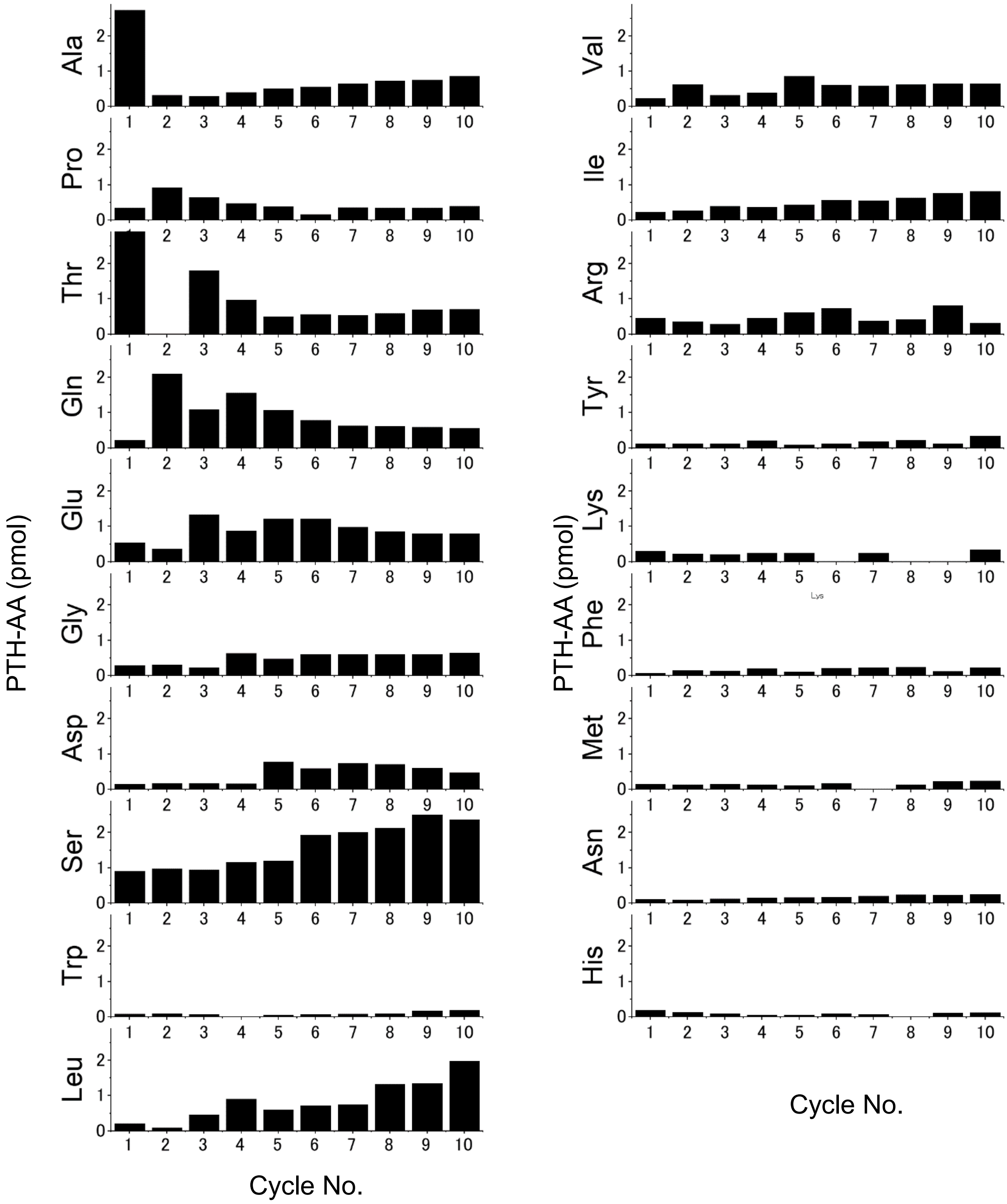

Fig. S2. Yields of PTH-AAs in Edman microsequencing cycles. Each chromatogram from protein sequencing (Fig. S1) was analyzed in SequencePro software (Applied Biosystems) to determine the yields of each PTH-AA. Yields of each PTH-AA over 10 cycles were depicted as bar graphs using OriginPro 2019 software (OriginLab Corporation). Possible protein sequences were determined by visual comparison between chromatograms of neighboring cycles overlayed on SequencePro as well as inspecting the changes in yields of each PTH-AA throughout the cycles, with background peaks and carry-over taken into account. As a result, one or more PTH-AAs were identified in each cycle: Residue 1: A and T, Residue 2: P and Q, Residue 3: T and E, Residue 4: Q and G and Residue 5: E and D, Residue 6: G and S, Residue 7: D, Residue 8: L, Residue 9: S, Residue 10: L. Cysteine was undetectable in this experiment because the sample was not treated with an alkylating reagent, and so was not shown in this figure.

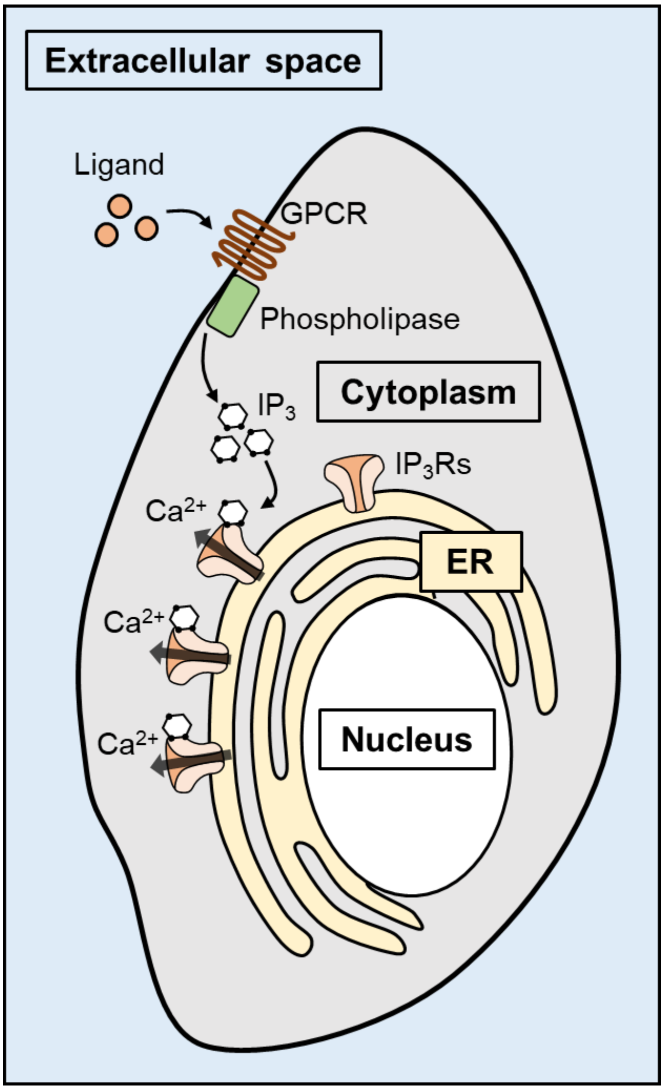

Fig. S3. Schematic diagram showing the Ca^{2+} signaling pathway upon GPCR stimulation. Once the ligands stimulate the GPCR, the IP_3 is produced by phospholipase. Subsequently, the IP_3 is bound with IP_3Rs, resulting in the Ca^{2+} release from the ER into the cytoplasm.

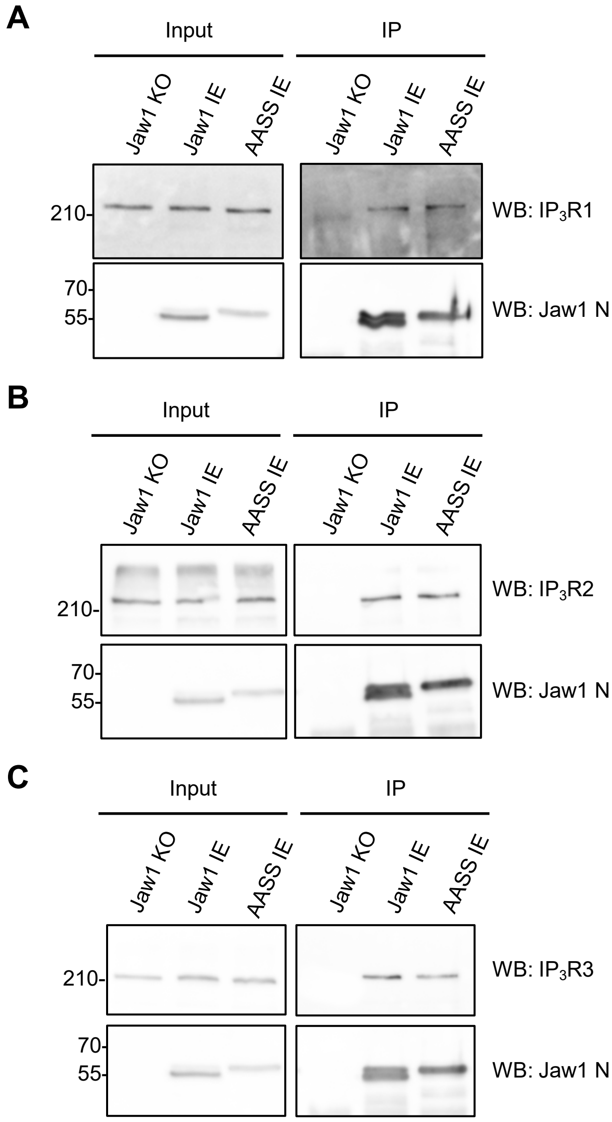

Fig. S4. Investigation into the relevance of the Jaw1 C-terminal cleavage event to its interaction with IP$_3$Rs. Jaw1 KO, Jaw1 IE, and AASS IE cells were treated with Dox for 24 h. The lysates were subjected to co-immunoprecipitation assay using an anti-Jaw1 N antibody followed by western blotting using an anti-IP3R1 antibody (A), an anti-IP3R2 antibody (B), and an anti-IP3R3 antibody (C) with an anti-Jaw1 N antibody. The representative blot images from two independent experiments with similar results are shown.

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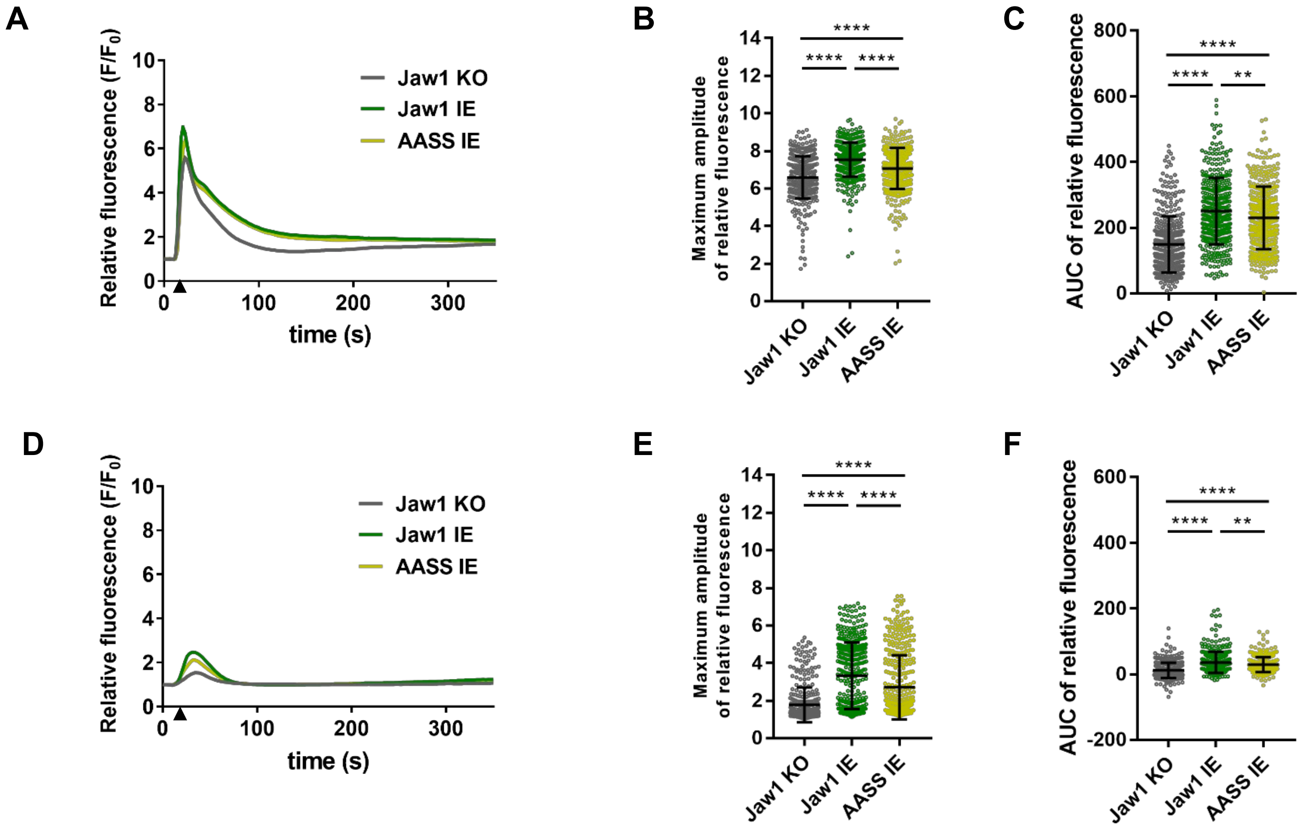

Fig. S5. Investigation into the relevance of the Jaw1 C-terminal cleavage event to the regulation of Ca^{2+} release via IP_3Rs. Jaw1 KO, Jaw1 IE, and AASS IE cells were treated with Dox for 24 h followed by Ca^{2+} imaging. The cells were stimulated with 5 µM (A–C) and 0.5 µM (D–F) ATP solution. A, D) Mean curves of relative Fluo-4 intensity. Closed triangles represent the time points that ATP solution was added. B, C, E, F) The maximum amplitude (B, E) and AUC (C, F) of relative Fluo-4 intensity (n = 400). B, C, E, F). Error bar shows ± SD, $^{**}P < 0.01$; $^{****}P < 0.0001$. Statistics: one-way ANOVA followed by Tukey's multiple comparison test.

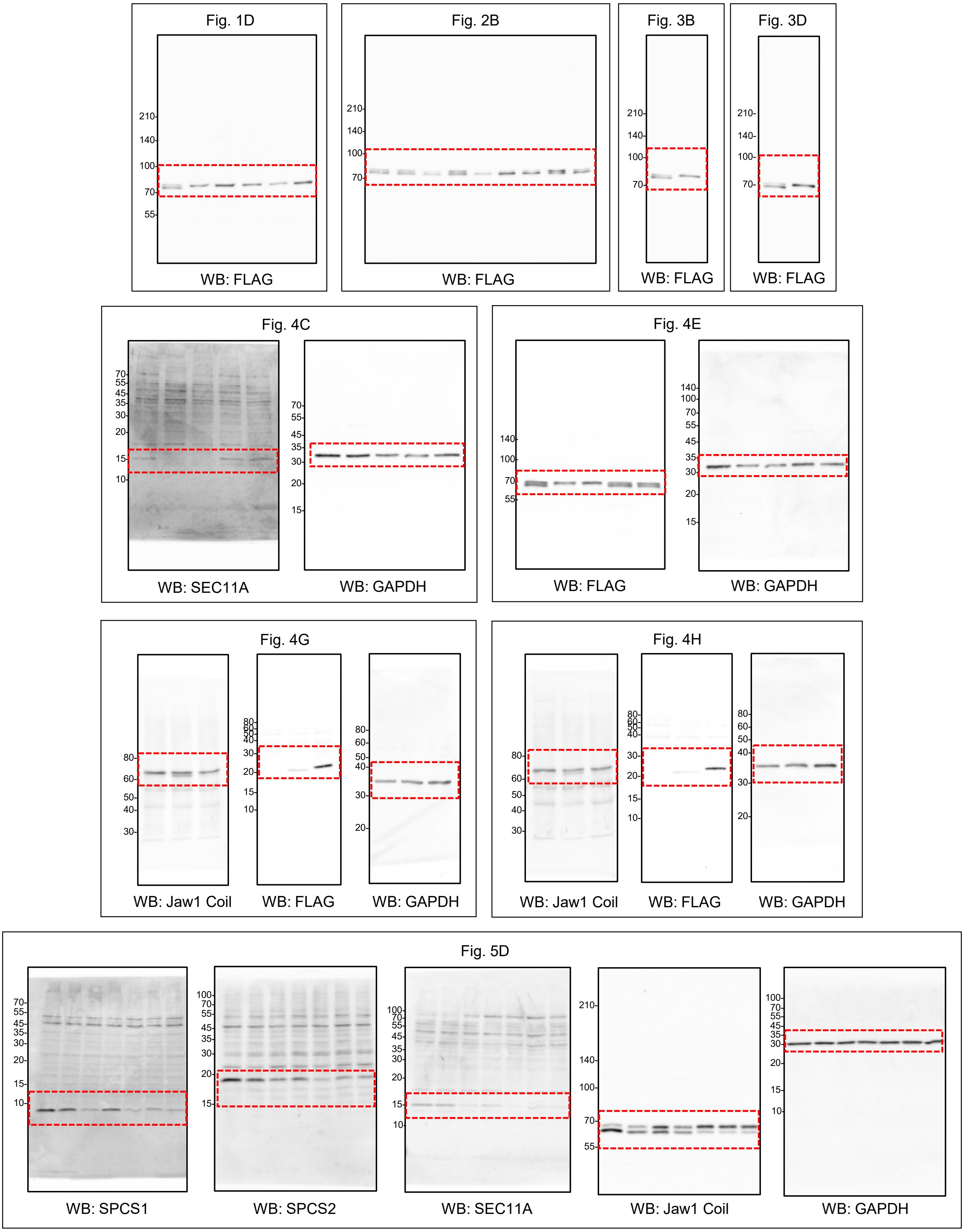

Fig. S6. Blot transparency. The area surrounded with red dot lines were cropped.

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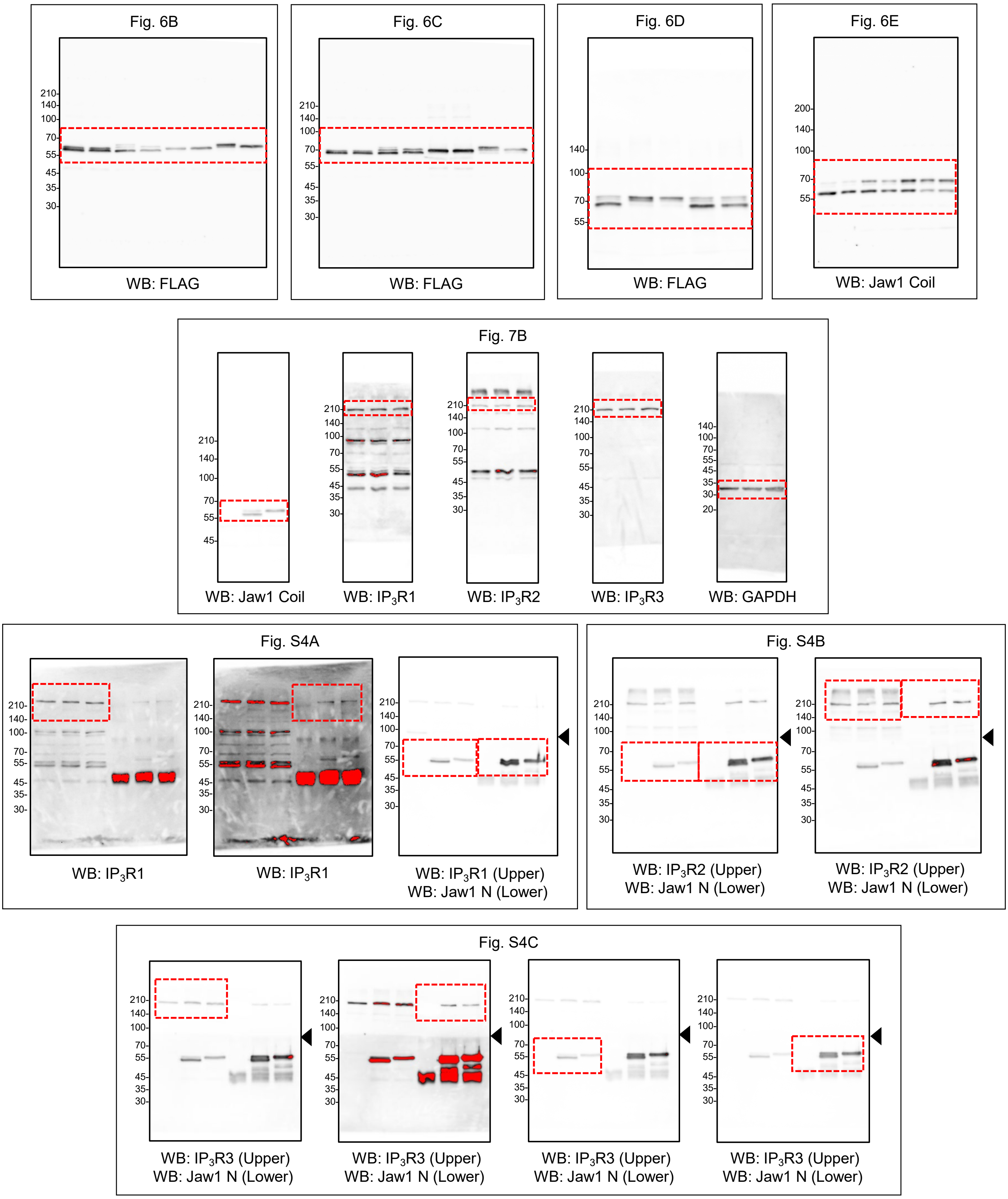

Fig. S7. Blot transparency. The area surrounded with red dot lines were cropped. Several membranes were cut at the black arrow point prior to hybridization with antibodies, thereby the full-length blots were not prepared.

Table S1. Primer sets to produce the plasmids used for this study

Number	Forward/Reverse	Sequence
Primer 1	Forward	5'-CATACTGATCGCAGCGCTGATGAGCTTCCTCACAGGTTCAGAGAAAGACTACAGCTGTGCCC-3'
Primer 1	Reverse	5'-CTGGACTAGTGGATCCTCAGAGTGGAGGAGGACCGTTGG-3'
Primer 2	Forward	5'-CATACTGATCGCAGCGCTGATGAGCTTCCTCACAGGTTCTGAAGATGACTACAGCTGCACCC-3'
Primer 2	Reverse	5'-TGGACTAGTGGATCCCTAGGTGGGAGGTGGCCCGTTG-3'
Primer 3	Forward	5'-CATACTGATCGCAGCGCTGATGAGCTTCCTCACAGGTGGCGAGGAGGAGCGC-3'
Primer 3	Reverse	5'-CTGGACTAGTGGATCCCTAGGTGGGCGGGGGGCCATTGTA-3'
Primer 4	Forward	5'-CATACTGATCGCAGCGCTGATGAGCTTCCTCACAGGTTCGGGGGTCTCCTGCT-3'
Primer 4	Reverse	5'-CTGGACTAGTGGATCCTCAGATTGGAGGGAGACCATTGACATAACTGAGC-3'
Primer 5	Forward	5'-CATACTGATCGCAGCGCTGATGAGCTTCCTCACAGGTAGCCAGTCCCCGCCA-3'
Primer 5	Reverse	5'-CTGGACTAGTGGATCCTCACACTGGTGGCGGCTGTAGGTA-3'
Primer 6	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 6	Reverse	5'-GGCCGCTGCAGCGGCGAAGAGCTGACCTGTGAGG-3'
Primer 7	Forward	5'-GCCGCTGCAGCGGCCGCTGCGCCCACACA-3'
Primer 7	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 8	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 8	Reverse	5'-GGCCGCTGCAGCGGCGGCTGTCTGGAAGAGCTG-3'
Primer 9	Forward	5'-GCCGCTGCAGCGGCCACACAGGAGGGGGACTC-3'
Primer 9	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 10	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 10	Reverse	5'-GGCCGCTGCAGCGGCGGGCGCAGCCTCCA-3'
Primer 11	Forward	5'-GCCGCTGCAGCGGCCTCCTGGCTGTCTCTAGAACA-3'
Primer 11	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 12	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 12	Reverse	5'-GGCCGCTGCAGCGGCGTCCCCCTCCTGTGTGG-3'
Primer 13	Forward	5'-GCCGCTGCAGCGGCCGAACACATCTTATGGCCATTTACCAG-3'
Primer 13	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 14	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 14	Reverse	5'-GGCCGCTGCAGCGGCTAGAGACAGCCAGGAGTCCC-3'
Primer 15	Forward	5'-GCCGCTGCAGCGGCCCCATTTACCAGACTCGGGCATG-3'
Primer 15	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 16	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 16	Reverse	5'-GGCCGCTGCAGCGGCCCATAAGATGTGTTCTAGAGACAGCC-3'
Primer 17	Forward	5'-GCCGCTGCAGCGGCCGGGCATGATGGACCACTG-3'
Primer 17	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 18	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 18	Reverse	5'-GGCCGCTGCAGCGGCGAGTCTGGTAAATGGCCATAAGATGTG-3'
Primer 19	Forward	5'-GCCGCTGCAGCGGCCCTGCCAGTGTGAGGATCCA-3'
Primer 19	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 20	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 20	Reverse	5'-GGCCGCTGCAGCGGCATCATGCCCGAGTCTGGT-3'
Primer 21	Forward	5'-GCCGCTGCAGCGGCCTGAGGATCCACTAGTCCAGT-3'
Primer 21	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 22	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 22	Reverse	5'-AGGGCTAGATTCGACGGCTGTCTGGAAGAGCTGA-3'
Primer 23	Forward	5'-GTCGAATCTAGCCCTACACAGGAGGGGGACTC-3'
Primer 23	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 24	Forward	5'-ACTGTGAAATCGGTTAACGTTAGACAAA-3'
Primer 24	Reverse	5'-CGGACTTGAGTCAACAGACTTCTGGAATAATTGGCCTGT-3'
Primer 25	Forward	5'-GTTGACTCAAGTCCGACACAGCAAGAGGACTCATGGA-3'
Primer 25	Reverse	5'-CCACCGCATCCCCAGGGTAACCCCATAGAGCCCACCGC-3'
Primer 26	Forward	5'-TCTGTGAAGCTCGTTAACTTTCAGC-3'
Primer 26	Reverse	5'-CTGGACTAGTGGATCCTCAGCCCGTCTTGTTGGAGAAAGGCACGTAGAAGTTTGGGCCCACTGGCAGTGGTCCATC-3'
Primer 27	Forward	5'-ACTGTGAAATCGGTTAACGTTAGACAAA-3'
Primer 27	Reverse	5'-GCCCTCTAGACTCGAGTCAGCCCGTCTTGTTGGAGAAAGGCACGTAGAAGTTTGGGCCCACTGGTGGTGGCCCA-3'
Primer 28	Forward	5'-TCGAGGGCGTGGCGATGCCGGGCGCGGAAGATGATGTGGTGGT-3'
Primer 28	Reverse	5'-CTAGACCACCACATCATCTTCCGCGCCCGGCATCGCCACGCCC-3'
Primer 29	Forward	5'-TTAAACTTAAGCTTGGTACCATGCTCTGTGTAA-3'
Primer 29	Reverse	5'-TCGCCACGCCCTCGAGCACTGGCAGTGGTCCATC-3'
Primer 30	Forward	5'-ACTGCCAGTGCTCGAGATGCTCTGTGTAAAAGGTCC-3'
Primer 30	Reverse	5'-TCGCCACGCCCTCGACCTGCTGAAAGTTAACGAGCT-3'
Primer 31	Forward	5'-CACCGAAGTGAAAGTCCGATTGTAG-3'
Primer 31	Reverse	5'-AAACCTACAATCGGACTTTCACTTC-3'
Primer 32	Forward	5'-CACCGAAAGGCTTGATCGTGCTCAC-3'
Primer 32	Reverse	5'-AAACGTGAGCACGATCAAGCCTTTC-3'
Primer 33	Forward	5'-TTTAAACTTAAGCTTGGTACCATGCTGTCTCTAGACTTTTTGGACG-3'
Primer 33	Reverse	5'-CCACACTGGACTAGTGGATCCTTACTCACGATGAACCAGCACG-3'
Primer 34	Forward	5'-TTTAAACTTAAGCTTGGTACCATGGTGCGTGCGGG-3'
Primer 34	Reverse	5'-CCACACTGGACTAGTGGATCCTTAGGATTCACGTTTTAGTAACACATATGCA-3'
Primer 35	Forward	5'-GCATCTCCAGCTTCACAGGT-3'
Primer 35	Reverse	5'-GCTCTCTCTGCCTGTTTGGT-3'

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