Supporting information

A guide-tag system controlling client enrichment into Y15 peptide-based granules for an in-cell protein recruitment assay

Takayuki Miki*, Masahiro Hashimoto, Taichi Nakai & Hisakazu Mihara

School of Life Science and Technology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa 226-8501, Japan

Contents

Materials and Methods S3

Table S1 Amino acid sequence of proteins used in this study S6

Table S2 Primer and oligo DNA sequences used in this study S14

Fig. S1 Confocal observation of COS-7 cells expressing Yn-mCh-HA S16

Fig. S2 Insoluble fraction of Yn-mCh-HA (client) in COS-7 cells expressing Y15-AG-myc S16

Fig. S3 Raw western blot images of pull-down assay of Yn-mCh-HA (client) in COS-7 cells expressing Y15-AG-myc or AG-myc (scaffold). S17

Fig. S4 Incorporation of various clients into the Y15-AG-myc scaffold. S18

Fig. S5 Confocal observation of COS-7 cells expressing Y15-AG-myc, mCh-Nck1(1-258) without guide-tag, and mTagBFP2-N-WASP. S19

Fig. S6 Confocal observation of COS-7 cells expressing Y15-AG-myc, Y15-mCh-HA without Nck1 bait protein, and mTagBFP2-N-WASP. S19

Fig. S7 Partition coefficients of bait Nck1(1-258) and prey N-WASP. S20

Fig. S8 Box plots correlating the relative expression level of Yn-mCh-Nck1(1-258) to mTagBFP2-N-WASP with the partition coefficient ratio. S20

Fig. S9 Effects of the relative expression level of mTagBFP2-N-WASP to Y11-mCh-Nck1(1-258) on the partition coefficients. S21

Fig. S10 Confocal images of COS-7 cells expressing mTagBFP2-SOS1(1014-1333), Yn-mCh-Grb2 and Y15-AG-myc. S22

Fig. S11 Partition coefficients of bait Grb2 and prey SOS1(1014-1333). S23

Fig. S12 FRAP measurements of mTagBFP2-N-WASP inside the granules. S24

Fig. S13 FRAP measurements of mTagBFP2-SOS1(1014-1333) inside the granules. S25

Reference S26

Materials and Methods

Construction of plasmid

The genes of SOD1, catalase, HSP70, STIP1, N-WASP, Grb2 and SOS1 were cloned by nested-PCR from Human Brain, whole Marathon®-Ready cDNA (Clontech, 639300). These genes were subcloned into pCI-neo vector (Promega). The gene of mTagBFP2 was subcloned from mTagBFP2-Lifeact-7, which was a gift from Michael Davidson (Addgene plasmid # 54602; http://n2t.net/addgene:54602; RRID: Addgene_54602). We purchased the oligo DNAs coding myc-tag or Yn peptides from FASMAC. The gene of Yn peptide was inserted between *NheI* site and *EcoRI* site. The gene of myc-tag was inserted between *SalI* site and *NotI* site. The other plasmids were constructed in the previous our report[1]. All protein sequence and all oligo DNA sequence are listed in Table S1 and S2, respectively.

Cell culture and transfection

COS-7 cell was provided by the RIKEN BRC through the National BioResource Project of the MEXT/AMED, Japan. The cell was cultured in Dulbecco's modified Eagles' medium (D-MEM, Wako) supplemented with 10% fetal bovine serium (Sigma) and 1% penicillin/streptomycin (Nacalai) in 5% CO_2 atmosphere at 37 ºC (MCO-5AC, PHC). COS-7 cells (1.5×10^5 or 1.5×10^4 cells) were seeded in a 24-well plate or a 96-well plate and incubated for 12-24 hours, followed by lipofection using Lipofectamine® 3000 (Thermo). The transfected cells were incubated for two days before experiments.

Sedimentation assay

The transfected cells were rinsed with PBS buffer, then lysed in Ripa buffer (100 μL) containing a 1% protease inhibitor cocktail (Nacalai) on ice. The lysate was centrifuged (16,000 × g, 10 min). The soluble fraction (80 μL) was collected. The pellet was rinsed with PBS buffer (200 μL), centrifuged (16,000 × g, 10 min) and removed supernatant. Soluble fraction was mixed with 2× Laemmli buffer (80 μL), and the pellet was dissolved in 1× Laemmli buffer (200 μL). Both fractions were boiled at 95 ºC for 10 min, then loaded onto SDS-PAGE (12.5% AA gel). The proteins in gels were transferred onto an Immobilon PVDF membrane (Millipore) by using Trans-Blot® SD Semi-Dry Transfer Cell (BioRad). The membrane was blocked with 1% skimmed milk in TBS-T for 30 min at room temperature, then incubated with 1% skimmed milk in TBS-T containing a rabbit anti-myc antibody (MBL, 562MS, 1:2,500 dilution) or a rabbit anti-HA antibody (MBL, 561, 1:2,500 dilution) for overnight at 4 ºC. The membrane was washed with TBS-T for three times, followed by incubation with 1% skimmed milk in TBS-T containing a goat anti-rabbit IgG HRP conjugate (Abcam, ab6721, 1:2,500 dilution) for 1 hour at room temperature. After washed with TBS-T for three times, working solution of ECL prime (Cytiva) was added, and the chemiluminescent bands were detected by LuminoGraph I instrument (ATTO) using ImageSaver6.

Pull-down assay

Two days after transfection, the COS-7 cells were lysed with 200 μL of 0.2% SDS Ripa buffer (50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1w/v% Nonidet P40 substitute, 0.5w/v% sodium deoxycholate, 0.2w/v% SDS) containing a 1% protease inhibitor cocktail (Nacalai) and sonicated for 5 min on ice. The protein concentration was determined by the BCA protein assay kit (TAKARA). For the preparation of anti-Myc antibody-coated magnetic beads, 150 μL of SureBeads Protein G (BioRad, #161-4021) were washed with TBS-T buffer (three times) and incubated with 3 μL of rabbit anti-Myc tag pAb (MBL, 562MS) for 10 min at room temperature. The beads were rinsed with 1 mL TBS-T (three times), then divided into six tubes. The lysates were diluted into TBS-T to 0.1 mg/mL proteins and subsequently mixed with the antibody-coated beads. After overnight incubation at 4 ºC with rotation, the beads were rinsed with TBS-T (three times), then the proteins were eluted by boiling the beads in 80 μL of 1× Laemmli buffer (100 mM DTT). The lysates before pull-down (Input) and eluted solutions (Output) were loaded to SDS-PAGE gels (12.5% AA) and transferred onto Immobilon PVDF membranes (Millipore). The membranes were blocked with 1% skimmed milk in TBS-T for 30 min at room temperature, then incubated with 1% skimmed milk in TBS-T containing a mouse anti-HA-tag mAb (MBL, M180-3, 1:2,500 dilution) for overnight at 4 ºC. After washing with TBS-T (three times), the membranes were incubated with a goat anti-mouse IgG HRP conjugate (Promega, W402B, 1:2,500 dilution) in 1% skimmed milk in TBS-T for 1 hour at room temperature. After washing with TBS-T three times, the membranes were soaked with the working solutions of ECL prime (Cytiva), and the chemiluminescent bands were detected by LuminoGraph I instrument (ATTO).

Fluorescence anisotropy measurement

The transfected cells were transferred on a 96-well plate and incubated for one day. After substitution of medium into D-MEM (HEPES, no Phenol Red, Wako), the fluorescence anisotropy was obtained by ARVO MX plate reader with Wallac1420 Workstation. The AG fluorescence was detected at excitation 485/14-nm (F485 filter) and emission 535/25-nm (F535 filter).

Fluorescence observation

The cells were transferred on glass-based dishes (Matsunami) or a glass-based 96-well plate (IWAKI) one day after transfection. The transfected cells were incubated for another one day, and the medium was substituted with D-MEM (HEPES, no Phenol Red, Wako) one hour before observation. Fluorescent cells were observed by using confocal laser fluorescence microscopy (LSM780, Zeiss) with ZEN2.3 (black edition) software. We used a 405-nm laser diode, a 488-nm argon laser and a 561-nm diode-pumped solid-state laser for exciting mTagBFP2, AG and mCh, respectively.

Image processing

The calculation of the partition coefficient (PC) was performed by using python 3.7 (Scikit-image 0.18.1) with the following steps: For the COS-7 cells expressing Y15-AG-myc and Yn-mCh-HA, we acquired two-channel images (channel 1: fluorescent image of AG, channel 2: fluorescent image of mCh). Initially, we identified the cell region and granular region by the 60 percentile-threshold of channel 2 and otsu-threshold of channel 1, respectively. The bulk region was defined as the area inside the cell and outside the granule. The fluorescence intensity of all pixels was subtracted by the 60-percentile-threshold (background signal). The PC value was calculated by dividing the mean fluorescence value in the granules divided by the mean fluorescence value in the bulk. For the COS-7 cells expressing Y15-AG-myc, Yn-mCh-bait and mTagBFP2-prey, three-channel data (channel 1: fluorescent image of AG, channel 2: fluorescent image of mCh, channel 3: fluorescent image of mTagBFP2) were obtained. The cell region was defined as the combined regions above the 70 percentile-thresholds of channel 2 and channel 3. Other steps are same as described above.

FRAP analysis

Two days after transfection, the medium was substituted with D-MEM (HEPES, no Phenol Red, Wako) one hour before observation. Fluorescence observation and FRAP measurements were conducted using confocal laser fluorescence microscopy (LSM780, Zeiss) with ZEN2.3 (black edition) software. For FRAP measurement, regions of interest (diameter of 1.3 μm) were bleached with 405-nm laser illumination (100% power). The images were acquired every 6 sec. Analyzing FRAP data was performed with ZEN2.3 (black edition) software. We set ROIs outside of bleached regions for reference. Curves were fit with a single exponential mode to determine the mobile fraction and the half-time.

Table S1. Amino acid sequence of proteins used in this study

Protein	Sequence	Reference
Y15-AG-myc	MYEYKYEYKYEYKYEYGGGEFMVSVIKPEMKIKLCMRGTV NGHNFVIEGEGKGNPYEGTQILDLNVTEGAPLPFAYDILTTVF QYGNRAFTKYPADIQDYFKQTFPEGYHWERSMTYEDQGICTA TSNISMRGDCFFYDIRFDGVNFPPNGPVMQKKTLKWEPSTEK MYVRDGVLKGDVNMALLLEGGGHYRCDFKTTYKAKKDVR LPDYHFVDHRIEILKHDKDYNKVKLYENAVARYSMLPSQAKV DEQKLISEEDL	Constructed in this work
mCh-HA	MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRP YEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADI PDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIK QRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITS HNEDYTIVEQYERAEGRHSTGGMDELYKVDYPYDVPDYAGY PYDVPDYA	1
Y9-mCh-HA	MYEYKYEYKYGGGEFMVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSP QFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGV VTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKP VQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYKVDYPYDVPDYAGYPYDVPDYA	Constructed in this work
Y11-mCh-HA	MYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFKVHM EGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDIL SPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGG VVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWE ASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKK PVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMD ELYKVDYPYDVPDYAGYPYDVPDYA	Constructed in this work
Y13-mCh-HA	MYEYKYEYKYEYKYGGGEFMVSKGEEDNMAIIKEFMRFKV HMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAW DILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFE DGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTM GWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYK AKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTG	Constructed in this work

	GMDELYKVDYPYDVPDYAGYPYDVPDYA	
Y15-mCh-HA	MYEYKYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKT MGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTT YKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKVDYPYDVPDYAGYPYDVPDYA	1
Y15-mCh-SOD1	MYEYKYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKT MGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTT YKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKVDMATKAVCVLKGDGPVQGIINFEQKESNGPV KVWGSIKGLTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKH GGPKDEERHVGDLGNVTADKDGVADVSIEDSVISLSGDHCIIG RTLVVHEKADDLGKGGNEESTKTGNAGSRLACGVIGIAQ	Constructed in this work
Y15-mCh-catalase	MYEYKYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKT MGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTT YKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKVDMADSRDPASDQMQHWKEQRAAQKADVLTT GAGNPVGDKLNVITVGPRGPLLVQDVVFTDEMAHFDRERIPE RVVHAKGAGAFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFS TVAGESGSADTVRDPRGFAVKFYTEDGNWDLVGNNTPIFFIRD PILFPSFIHSQKRNPQTHLKDPDMVWDFWSLRPESLHQVSFLF SDRGIPDGHRHMNGYGSHTFKLVNANGEAVYCKFHYKTDQG IKNLSVEDAARLSQEDPDYGIRDLFNAIATGKYPSWTFYIQVM TFNQAETFPFNPFDLTKVWPHKDYPLIPVGKLVLNRNPVNYFA EVEQIAFDPSNMPPGIEASPDKMLQGRLFAYPDTHRHRLGPNY LHIPVNCPYRARVANYQRDGPMCMQDNQGGAPNYYPNSFGA PEQQPSALEHSIQYSGEVRRFNTANDDNVTQVRAFYVNVLNE EQRKRLCENIAGHLKDAQIFIQKKAVKNFTEVHPDYGSHIQAL	Constructed in this work

	LDKYNAEKPKNAIHTFVQSGSHLAAREKADL	
Y15-mCh-HSP70	MYEYKYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKT MGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTT YKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKVDMAKAAAIGIDLGTTYSCVGVFQHGKVEIIAN DQGNRTTPSYVAFTDTERLIGDAAKNQVALNPQNTVFDAKRL IGRKFGDPVVQSDMKHWPFQVINDGDKPKVQVSYKGDTKAF YPEEISSMVLTKMKEIAEAYLGYPVTNAVITVPAYFNDSQRQA TKDAGVIAGLNVLRIINEPTAAAIAYGLDRTGKGERNVLIFDL GGGTFDVSILTIDDGIFEVKATAGDTHLGGEDFDNRLVNHFVE EFKRKHKKDISQNKRAVRRLRTACERAKRTLSSSTQASLEIDS LFEGIDFYTSITRARFEELCSDLFRSTLEPVEKALRDAKLDKAQ IHDLVLVGGSTRIPKVQKLLQDFFNGRDLNKSINPDEAVAYGA AVQAAILMGDKSENVQDLLLLDVAPLSLGLETAGGVMTALIK RNSTIPTKQTQIFTTYSDNQPGVLIQVYEGERAMTKDNNLLGR FELSGIPPAPRGVPQIEVTFDIDANGILNVTATDKSTGKANKITI TNDKGRLSKEEIERMVQEAEKYKAEDEVQRERVSAKNALES YAFNMKSAVEDEGLKGKISEADKKKVLDKCQEVISWLDANT LAEKDEFEHKRKELEQVCNPIISGLYQGAGGPGPGGFGAQGP KGGSGSGPTIEEVD	Constructed in this work
Y15-mCh-STIP1	MYEYKYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKT MGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTT YKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKVEMEQVNELKEKGNKALSVGNIDDALQCYSEA IKLDPHNHVLYSNRSAAYAKKGDYQKAYEDGCKTVDLKPDW GKGYSRKAAALEFLNRFEEAKRTYEEGLKHEANNPQLKEGL QNMEARLAERKFMNPFNMPNLYQKLESDPRTRTLLSDPTYRE LIEQLRNKPSDLGTKLQDPRIMTTLSVLLGVDLGSMDEEEEIA TPPPPPPPKKETKPEPMEEDLPENKKQALKEKELGNDAYKKK DFDTALKHYDKAKELDPTNMTYITNQAAVYFEKGDYNKCRE	Constructed in this work

	LCEKAIEVGRENREDYRQIAKAYARIGNSYFKEEKYKDAIHFY NKSLAEHRTPDVLKKCQQAEKILKEQERLAYINPDLALEEKN KGNECFQKGDYPQAMKHYTEAIKRNPKDAKLYSNRAACYTK LLEFQLALKDCEECIQLEPTFIKGYTRKAAALEAMKDYTKAM DVYQKALDLDSSCKEAADGYQRCMMAQYNRHDSPEDVKRR AMADPEVQQIMSDPAMRLILEQMQKDPQALSEHLKNPVIAQK IQKLMDVGLIAIR	
mCh-Nck1(1-258)	MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRP YEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADI PDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIK QRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITS HNEDYTIVEQYERAEGRHSTGGMDELYKVDMAEEVVVVAKF DYVAQQEQELDIKKNERLWLLDDSKSWWRVRNSMNKTGFVP SNYVERKNSARKASIVKNLKDTLGIGKVKRKPSVPDSASPAD DSFVDPGERLYDLNMPAYVKFNYMAEREDELSLIKGTKVIVM EKCSDGWWRGSYNGQVGWFPSNYVTEEGDSPLGDHVGSLSE KLAAVVNNLNTGQVLHVVQALYPFSSSNDEELNFEKGDVMD VIEKPENDPEWWKCRKINGMVGLVPKNYVTVMQNNPLTSG	1
Y9-mCh-Nck1(1-258)	MYEYKYEYKYGGGEFMVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSP QFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGV VTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKP VQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYKVDMAEEVVVVAKFDYVAQQEQELDIKKNERLWLLDDSK SWWRVRNSMNKTGFVPSNYVERKNSARKASIVKNLKDTLGI GKVKRKPSVPDSASPADDSFVDPGERLYDLNMPAYVKFNYM AEREDELSLIKGTKVIVMEKCSDGWWRGSYNGQVGWFPSNY VTEEGDSPLGDHVGSLSEKLAAVVNNLNTGQVLHVVQALYPF SSSNDEELNFEKGDVMDVIEKPENDPEWWKCRKINGMVGLV PKNYVTVMQNNPLTSG	Constructed in this work
Y11-mCh-Nck1(1-258)	MYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFKVHM EGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDIL SPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGG VVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWE	Constructed in this work

	ASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKK PVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMD ELYKVDMAEEVVVVAKFDYVAQQEQELDIKKNERLWLLDDS KSWWRVRNSMNKTGFVPSNYVERKNSARKASIVKNLKDTLG IGKVKRKPSVPDSASPADDSFVDPGERLYDLNMPAYVKFNYM AEREDELSLIKGTKVIVMEKCSDGWWRGSYNGQVGWFPSNY VTEEGDSPLGDHVGSLSEKLAAVVNNLNTGQVLHVVQALYPF SSSNDEELNFEKGDVMDVIEKPENDPEWWKCRKINGMVGLV PKNYVTVMQNNPLTSG	
Y13-mCh-Nck1(1-258)	MYEYKYEYKYEYKYGGGEFMVSKGEEDNMAIIKEFMRFKV HMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAW DILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFE DGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTM GWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYK AKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTG GMDELYKVDMAEEVVVVAKFDYVAQQEQELDIKKNERLWLL DDSKSWWRVRNSMNKTGFVPSNYVERKNSARKASIVKNLKD TLGIGKVKRKPSVPDSASPADDSFVDPGERLYDLNMPAYVKF NYMAEREDELSLIKGTKVIVMEKCSDGWWRGSYNGQVGWF PSNYVTEEGDSPLGDHVGSLSEKLAAVVNNLNTGQVLHVVQ ALYPFSSSNDEELNFEKGDVMDVIEKPENDPEWWKCRKINGM VGLVPKNYVTVMQNNPLTSG	Constructed in this work
Y15-mCh-Nck1(1-258)	MYEYKYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKT MGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTT YKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKVDMAEEVVVVAKFDYVAQQEQELDIKKNERL WLLDDSKSWWRVRNSMNKTGFVPSNYVERKNSARKASIVK NLKDTLGIGKVKRKPSVPDSASPADDSFVDPGERLYDLNMPA YVKFNYMAEREDELSLIKGTKVIVMEKCSDGWWRGSYNGQ VGWFPSNYVTEEGDSPLGDHVGSLSEKLAAVVNNLNTGQVL HVVQALYPFSSSNDEELNFEKGDVMDVIEKPENDPEWWKCR KINGMVGLVPKNYVTVMQNNPLTSG	1
mTagBFP2-N-	MVSKGEELIKENMHMKLYMEGTVDNHHFKCTSEGEGKPYEG	Constructed

WASP	TQTMRIKVVEGGPLPFAFDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQDTSLQDGCLIYNVKIRGV NFTSNGPVMQKKTLGWEAFTETLYPADGGLEGRNDMALKLV GGSHLIANAKTTYRSKKPAKNLKMPGVYYVDYRLERIKEAN NETYVEQHEVAVARYCDLPSKLGHKLNVEMSSVQQQPPPPRR VTNVGSLLLTPQENESLFTFLGKKCVTMSSAVVQLYAADRNC MWSKKCSGVACLVKDNPQRSYFLRIFDIKDGKLLWEQELYNN FVYNSPRGYFHTFAGDTCQVALNFANEEEAKKFRKAVTDLLG RRQRKSEKRRDPPNGPNLPMATVDIKNPEITTNRFYGPQVNNI SHTKEKKKGKAKKKRLTKADIGTPSNFQHIGHVGWDPNTGF DLNNLDPELKNLFDMCGISEAQLKDRETSKVIYDFIEKTGGVE AVKNELRRQAPPPPPSRGGPPPPPPPPHNSGPPPPPARGRGAP PPPPSRAPTAAPPPPPPSRPSVAVPPPPPNRMYPPPPPALPSSAPS GPPPPPPSVLGVGPVAPPPPPPPPPPGPPPPPGLPSDGDHQVPT TAGNKAALLDQIREGAQLKKVEQNSRPVSCSGRDALLDQIRQ GIQLKSVADGQESTPPTPAPTSGIVGALMEVMQKRSKAIHSSD EDEDEDDEEDFEDDDEWED	in this work
mCh-Grb2	MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRP YEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADI PDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYK VKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIK QRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITS HNEDYTIVEQYERAEGRHSTGGMDELYKVDMEAIAKYDFKA TADDELSFKRGDILKVLNEECDQNWYKAELNGKDGFIPKNYI EMKPHPWFFGKIPRAKAEEMLSKQRHDGAFLIRESESAPGDFS LSVKFGNDVQHFKVLRDGAGKYFLWVVKFNSLNELVDYHRS TSVSRNQQIFLRDIEQVPQQPTYVQALFDFDPQEDGELGFRRG DFIHVMDNSDPNWWKGACHGQTGMFPRNYVTPVNRNV	Constructed in this work
Y9-mCh-Grb2	MYEYKYEYKYGGGEFMVSKGEEDNMAIIKEFMRFKVHMEG SVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSP QFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGV VTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKP VQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYKVDMEAIAKYDFKATADDELSFKRGDILKVLNEECDQNW YKAELNGKDGFIPKNYIEMKPHPWFFGKIPRAKAEEMLSKQR	Constructed in this work

	HDGAFLIRESESAPGDFSLSVKFGNDVQHFKVLRDGAGKYFL WVVKFNSLNELVDYHRSTSVSRNQQIFLRDIEQVPQQPTYVQ ALFDFDPQEDGELGFRRGDFIHVMDNSDPNWWKGACHGQT GMFPRNYVTPVNRNV	
Y11-mCh-Grb2	MYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFKVHM EGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDIL SPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGG VVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWE ASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKK PVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMD ELYKVDMEAIAKYDFKATADDELSFKRGDILKVLNEECDQNW YKAELNGKDGFIPKNYIEMKPHPWFFGKIPRAKAEEMLSKQR HDGAFLIRESESAPGDFSLSVKFGNDVQHFKVLRDGAGKYFL WVVKFNSLNELVDYHRSTSVSRNQQIFLRDIEQVPQQPTYVQ ALFDFDPQEDGELGFRRGDFIHVMDNSDPNWWKGACHGQT GMFPRNYVTPVNRNV	Constructed in this work
Y13-mCh-Grb2	MYEYKYEYKYEYKYGGGEFMVSKGEEDNMAIIKEFMRFKV HMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAW DILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFE DGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTM GWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYK AKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTG GMDELYKVDMEAIAKYDFKATADDELSFKRGDILKVLNEECD QNWYKAELNGKDGFIPKNYIEMKPHPWFFGKIPRAKAEEMLS KQRHDGAFLIRESESAPGDFSLSVKFGNDVQHFKVLRDGAGK YFLWVVKFNSLNELVDYHRSTSVSRNQQIFLRDIEQVPQQPTY VQALFDFDPQEDGELGFRRGDFIHVMDNSDPNWWKGACHG QTGMFPRNYVTPVNRNV	Constructed in this work
Y15-mCh-Grb2	MYEYKYEYKYEYKYEYGGGEFMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNF EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKT MGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTT YKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHS TGGMDELYKVDMEAIAKYDFKATADDELSFKRGDILKVLNEE CDQNWYKAELNGKDGFIPKNYIEMKPHPWFFGKIPRAKAEE	Constructed in this work

	MLSKQRHDGAFLIRESESAPGDFSLSVKFGNDVQHFKVLRDG AGKYFLWVVKFNSLNELVDYHRSTSVSRNQQIFLRDIEQVPQ QPTYVQALFDFDPQEDGELGFRRGDFIHVMDNSDPNWWKGA CHGQTGMFPRNYVTPVNRNV	
mTagBFP2- SOS1(1014-1333)	MVSKGEELIKENMHMKLYMEGTVDNHHFKCTSEGEGKPYEG TQTMRIKVVEGGPLPFAFDILATSFLYGSKTFINHTQGIPDFFK QSFPEGFTWERVTTYEDGGVLTATQDTSLQDGCLIYNVKIRGV NFTSNGPVMQKKTLGWEAFTETLYPADGGLEGRNDMALKLV GGSHLIANAKTTYRSKKPAKNLKMPGVYYVDYRLERIKEAN NETYVEQHEVAVARYCDLPSKLGHKLNVDLEIEPRNPKPLPRF PKKYSYPLKSPGVRPSNPRPGTMRHPTPLQQEPRKISYSRIPES ETESTASAPNSPRTPLTPPPASGASSTTDVCSVFDSDHSSPFHSS NDTVFIQVTLPHGPRSASVSSISLTKGTDEVPVPPPVPPRRRPES APAESSPSKIMSKHLDSPPAIPPRQPTSKAYSPRYSISDRTSISDP PESPPLLPPREPVRTPDVFSSSPLHLQPPPLGKKSDHGNAFFPN SPSPFTPPPPQTPSPHGTRRHLPSPPLTQEVDLHSIAGPPVPPRQ STSQHIPKLPPKTYKREHTHPSMHRDGPPLLENAHSS	Constructed in this work

Table S2. Primer and oligo DNA sequences used in this study

Protein	Fragment	Forward Sequence	Reverse Sequence
SOD1	1st PCR primer	gtttccgttgcagtcctcggaac	ggcctcagactacatccaaggg
	2nd PCR primer	gaagtcgacatggcgacgaaggccgtg	cttgcggccgcttattgggcgatcccaatt
Catalase	1st PCR primer	ctgcagtgttctgcacagcaaac	gctaagcttcgctgcacaggtg
	2nd PCR primer	gaagtcgacatggctgacagccgggatc	cttgcggccgctcacagatctgccttctcc
HSP70	1st PCR primer	gagccgacagagagcagggaac	ggaaatgcaaagtcttgaagctcc
	2nd PCR primer	ggagtcgacatggccaaagccgcg	gagcggccgcctaatctacctcctcaatg
STIP1	1st PCR primer	cggacggattcgattcaacg	cacatgagggcgaagggaag
	2nd PCR primer	gactcgagatggagcaggtcaatgagc	gagcggccgctcaccgaattgcaatcag
N-WASP	1st PCR primer	gagagggggaacgagctctc	ccacagacagaaagtagtgtttag
	2nd PCR primer	gaactcgagatgagctccgtccagcagc	cttgcggccgctcagtcttcccactcatcatc
Grb2	1st PCR primer	caggctgctgagcactgagcagcgct	cactttctttaaataattgcttcttg
	2nd PCR primer	caagtcgacatggaagccatcgccaaatat	gttgcggccgctcagacgttccggttcacgg gggt
SOS1(1014-1333)	1st PCR primer	ctctccccgcccagaggcgccccgg	ggaaaatatacatcccagtacagag
	2nd PCR primer	caagtcgacctagaaatagaaccacgaaac	gttgcggccgctcaggaagaatgggcattct ccaa
Myc-tag		tcgacgaacaaaaactcatctcagaagagga tctgtgagc	ggccgctcacagatcctcttctgagatgagttt ttgttcg
Y9	F1	ctagcgccaccatgtacgaatataaatacg	tattcgtatttatattcgtacatggtggcg
	F2	aatataaatacggcggtggcg	aattcgccaccgccgtattta
Y11	F1	ctagcgccaccatgtacgaatataaatacg	tattcgtatttatattcgtacatggtggcg

	F2	aatataaatacgaatatggcggtggcg	aattcgccaccgccatattcgtattta
Y13	F1	ctagcgccaccatgtacgaatataaatacg	tattcgtatttatattcgtacatggtggcg
	F2	aatataaatacgaatataaatacggcggtggcg	aattcgccaccgccgtatttatattcgtattta
Y15	F1	ctagcgccaccatgtacgaatataaatacg	tattcgtatttatattcgtacatggtggcg
	F2	aatataaatacgaatataaatacgaatatggcggtggcg	aattcgccaccgccatattcgtatttatattcgtattta

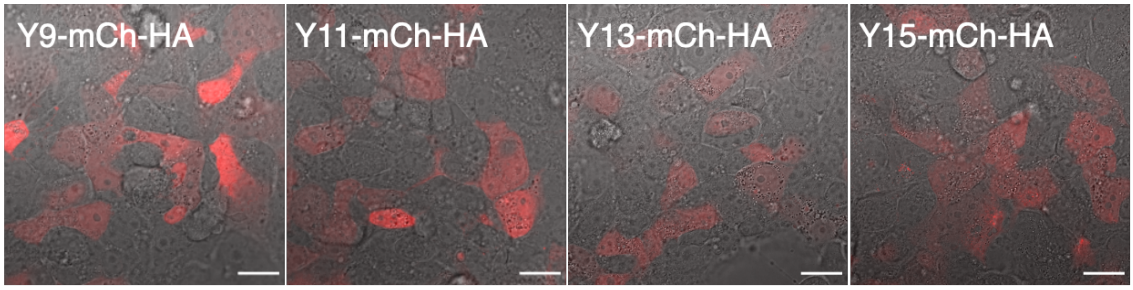

Fig. S1 Confocal observation of COS-7 cells expressing Yn-mCh-HA. Yn-mCh-HA protein dispersed in whole cells. Scale bar means 20 μm.

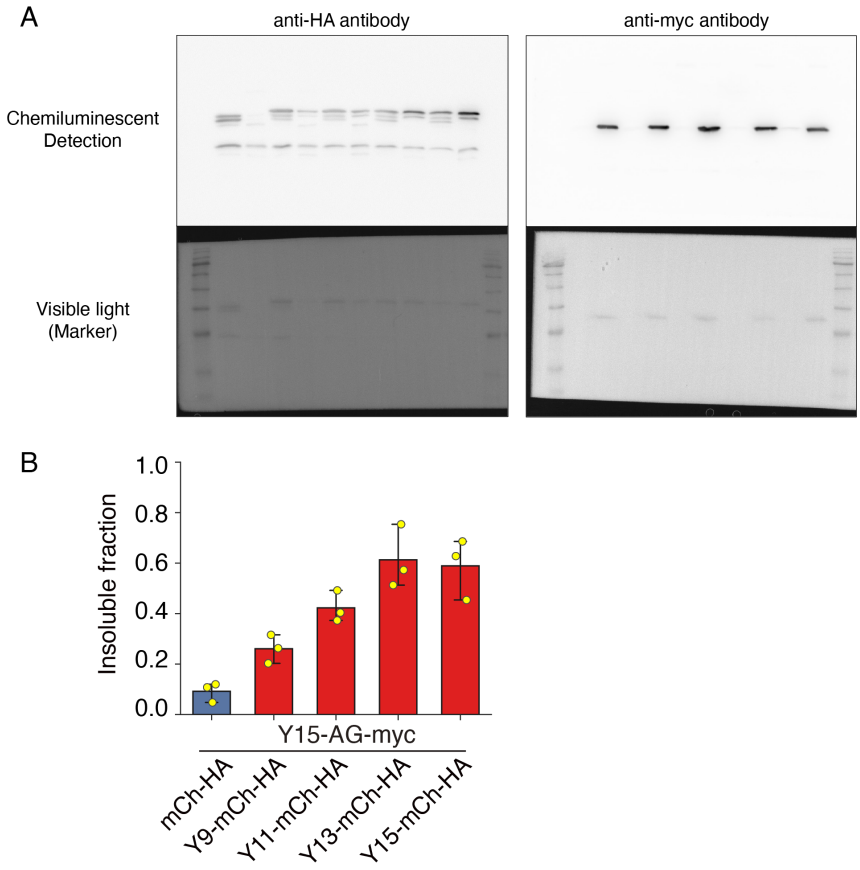

Fig. S2 Insoluble fraction of Yn-mCh-HA (client) in COS-7 cells expressing Y15-AG-myc. COS-7 cells expressing Y15-AG-myc and Yn-mCh-HA were lysed in Ripa buffer. The lysates were centrifuged and separated into soluble and insoluble fraction. (A) Raw western blot images of the soluble and insoluble fractions of cell lysates. (B) The insoluble fractions were quantified from western blotting results. Data are presented as mean values +/- S.D. (n = 3 biologically independent experiments).

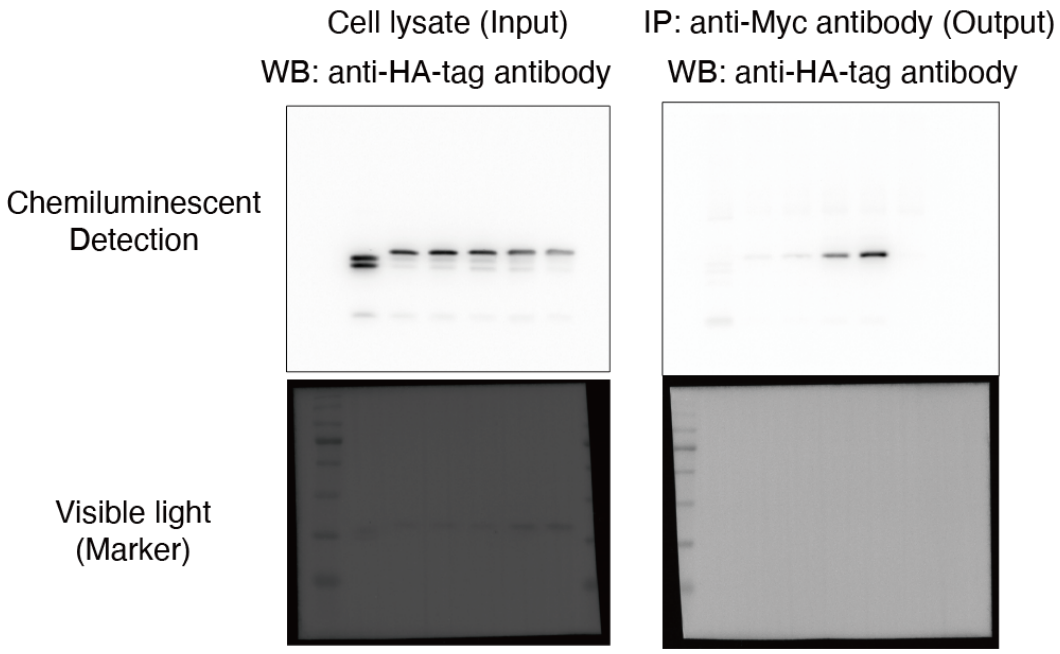

Fig. S3 Raw western blot images of pull-down assay of Yn-mCh-HA (client) in COS-7 cells expressing Y15-AG-myc or AG-myc (scaffold). The western blot image of cell lysates is shown in the left panel, and the image of eluted samples is shown in the right panel.

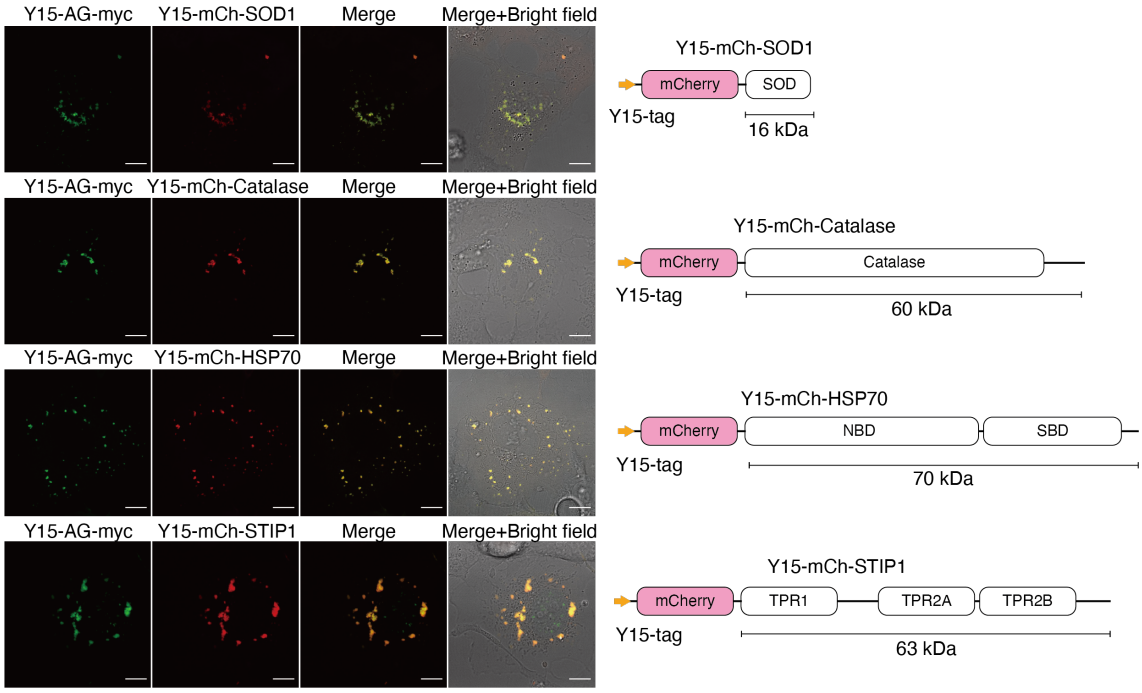

Fig. S4 Incorporation of various clients into the Y15-AG-myc scaffold. To demonstrate the client generality, the following four structurally distinct proteins were selected. SOD1; a copper/zinc superoxide dismutase 1 (16 kDa) [2], Catalase; an heme-containing globular enzyme (60 kDa) [3], HSP70; Heat shock 70 kDa protein 1A composed of a N-terminal nucleotide binding domain (NBD) and a C-terminal substrate-binding domain (SBD) (70 kDa) [4], STIP1; Stress-induced-phosphoprotein 1 containing three of TPR(tetratrico-peptide repeat) domains (63 kDa) [5]. The fluorescence images are shown in left panels, and the organization of Y15-tagging client proteins are shown in right panels. Scale bar means 10 μm. These images clearly indicate the colocalization of client proteins with the Y15-AG-myc scaffold.

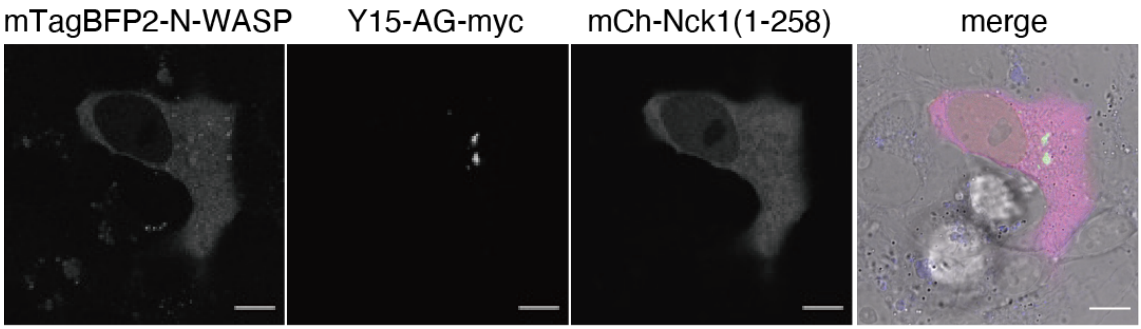

Fig. S5 Confocal observation of COS-7 cells expressing Y15-AG-myc, mCh-Nck1(1-258) without guide-tag, and mTagBFP2-N-WASP. Both mCh-Nck1(1-258) and mTagBFP2-N-WASP distributed in whole cells. Scale bar means 10 μm.

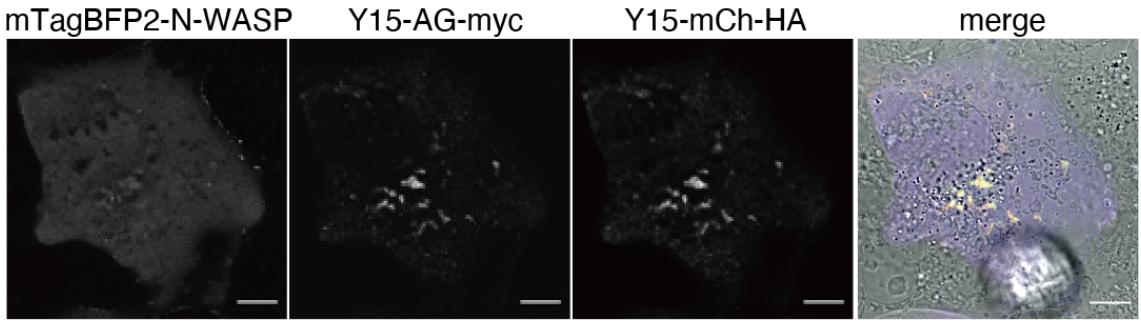

Fig. S6 Confocal observation of COS-7 cells expressing Y15-AG-myc, Y15-mCh-HA without Nck1 bait protein, and mTagBFP2-N-WASP. The mTagBFP2-N-WASP (prey protein) was distributed in cells when the Nck1 protein (bait) was substituted with an HA-tag. Scale bar means 10 μm.

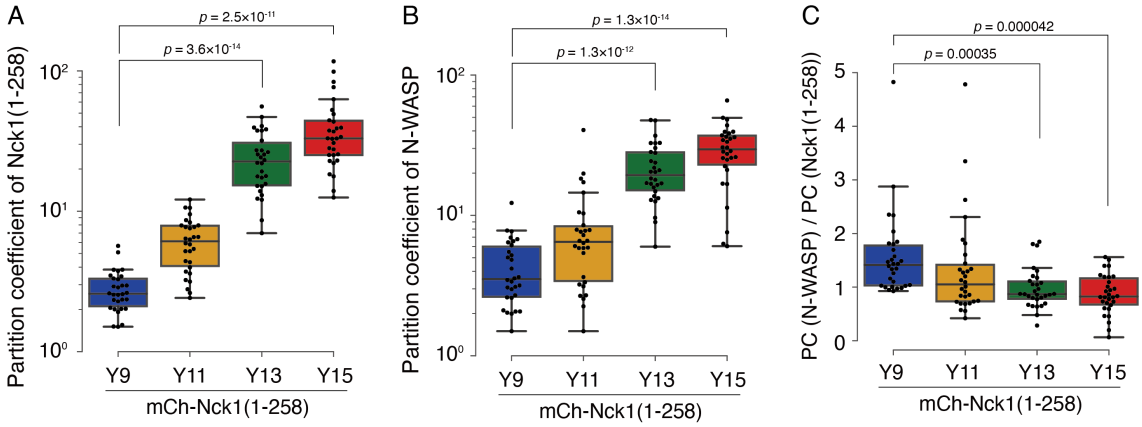

Fig. S7 Partition coefficients of bait Nck1(1-258) and prey N-WASP. (A-B) Box plots of PCs of (A) Yn-mCh-Nck1(1-258) and (B) mTagBFP2-N-WASP. As the length of the guide-tag increasing, both PCs gradually increased. (C) A box plot of PC ratio (N-WASP versus Nck1(1-258)). At the Y9-tag fusion, the prey N-WASP enriched in granules more than bait. These boxplots are presented with the elements: center line, median; box limits, Q1 and Q3; whiskers, 1.5× interquartile range; points, outliers. *p*-values; two-tailed paired t-tests.

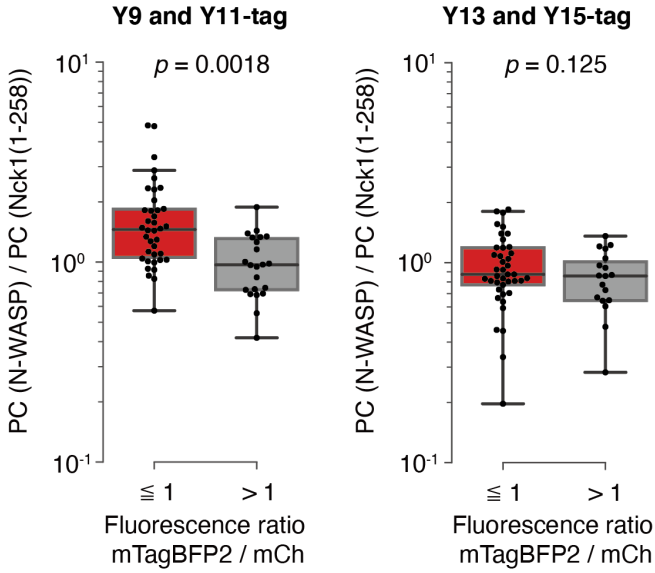

Fig. S8 Box plots correlating the relative expression level of mTagBFP2-N-WASP to Yn-mCh-Nck1(1-258) with the partition coefficient ratio. In the case of the Y9 and Y11 tags (low PC of the bait), the PC ratio tended to decrease when the relative expression of the prey to the bait was higher. On the other hand, in the case of the Y13 and Y15 tags, there was no clear difference in the PC ratio. These boxplots are presented with the elements: center line, median; box limits, Q1 and Q3; whiskers, 1.5× interquartile range; points, outliers. *p*-values; two-tailed paired t-tests.

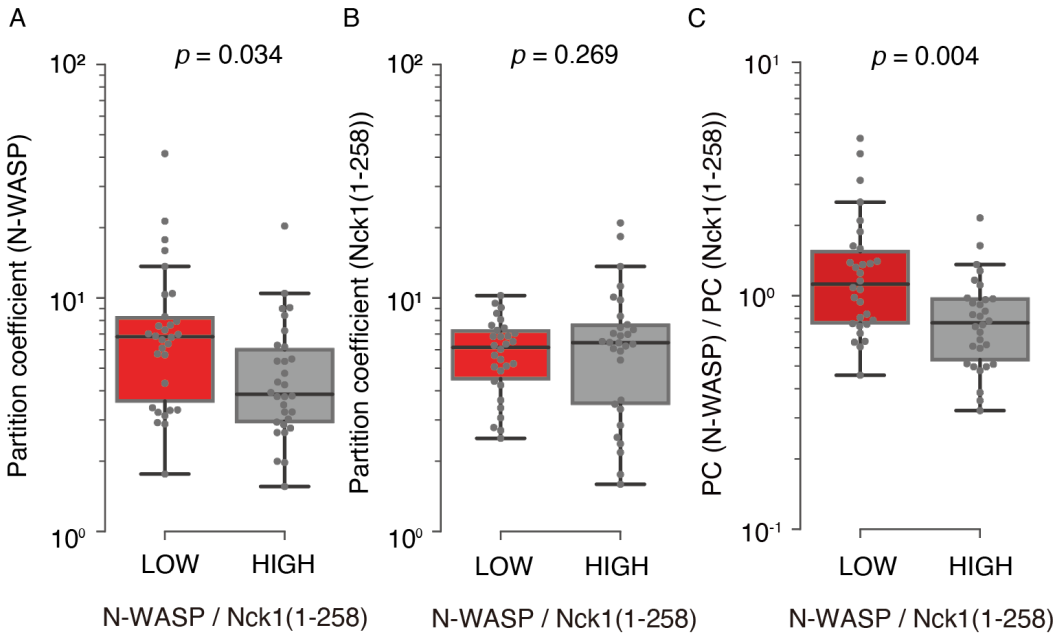

Fig. S9 Effects of the relative expression level of mTagBFP2-N-WASP to Y11-mCh-Nck1(1-258) on the partition coefficients. COS-7 cells (1.5×10^5 cells) were transfected with plasmid mixture in two different conditions (LOW (red); 250 ng/200 ng/50 ng, HIGH (gray); 250 ng/125 ng/125 ng for plasmid coding Y15-AG-myc/Y11-mCh-Nck1(1-258)/mTagBFP2-N-WASP, respectively). The partition coefficient of mTagBFP2-N-WASP (A) exhibits low value in the case of HIGH condition, while the value of Y11-mCh-Nck1(1-258) (B) was almost constant. The ratio of the partition coefficient (mTagBFP2-N-WASP/Y11-mCh-Nck1(1-258)) in the LOW condition (C) shows a significantly higher value than that in the HIGH condition. All boxplots are presented with the elements: center line, median; box limits, Q1 and Q3; whiskers, 1.5× interquartile range; points, outliers. (n = 30 cells over three biologically independent experiments). p-values; two-tailed paired t-tests.

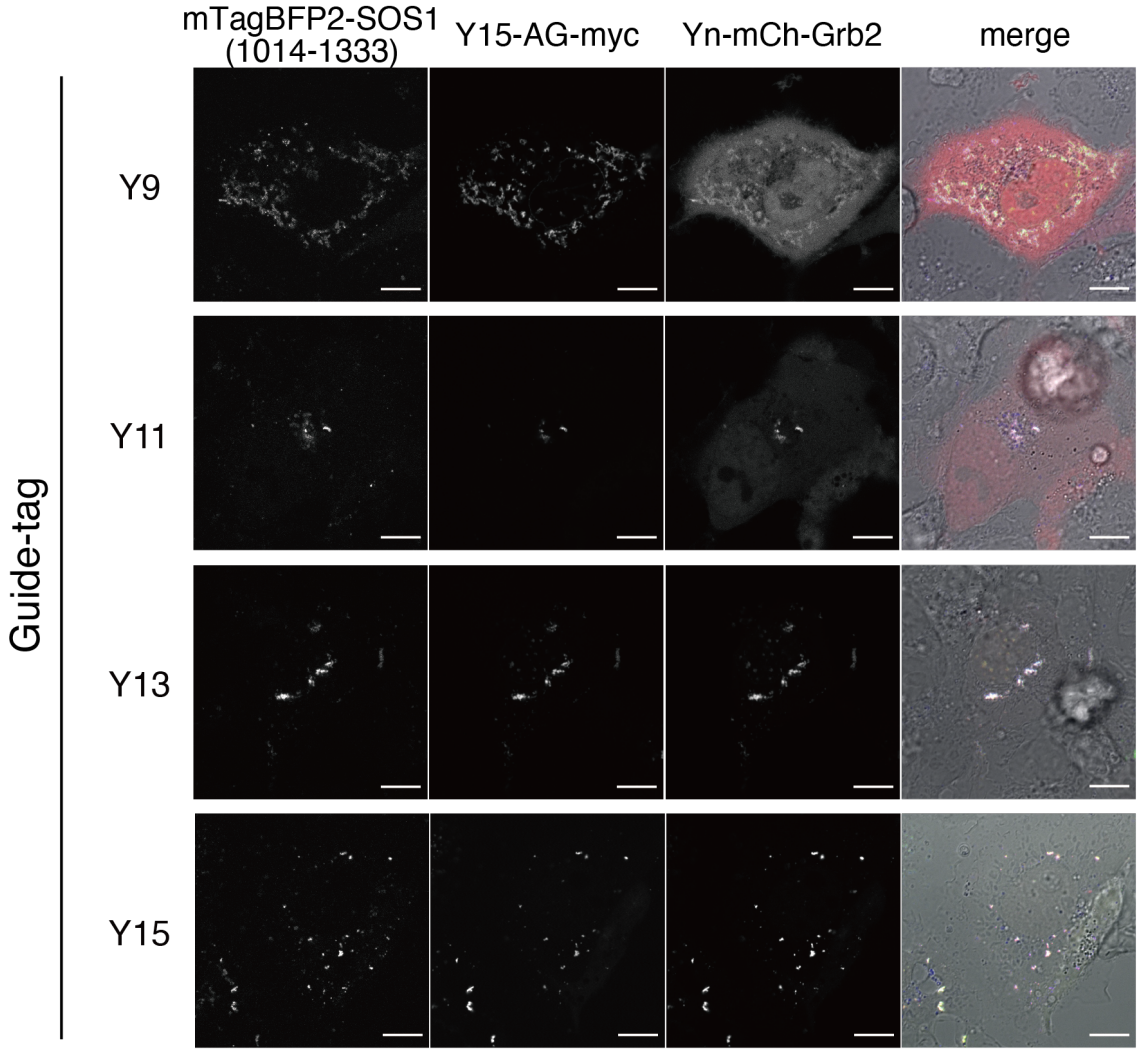

Fig. S10 Confocal images of COS-7 cells expressing mTagBFP2-SOS1(1014-1333), Yn-mCh-Grb2 and Y15-AG-myc. The COS-7 cells (2×10^4 cells) on a glass-based 96-well plate (IWAKI) were transfected with plasmid mixture (5/3/2 ratio for plasmid coding Y15-AG-myc/Yn-mCh-Grb2/ mTagBFP2-SOS1(1014-1333), respectively). The fluorescence images were obtained two days after transfection. Scale bar means 10 μm. At Y9-tagging, the major part of bait Grb2 was observed from the bulk, whereas the prey mTagBFP2-SOS1(1014-1333) was highly concentrated in the granules.

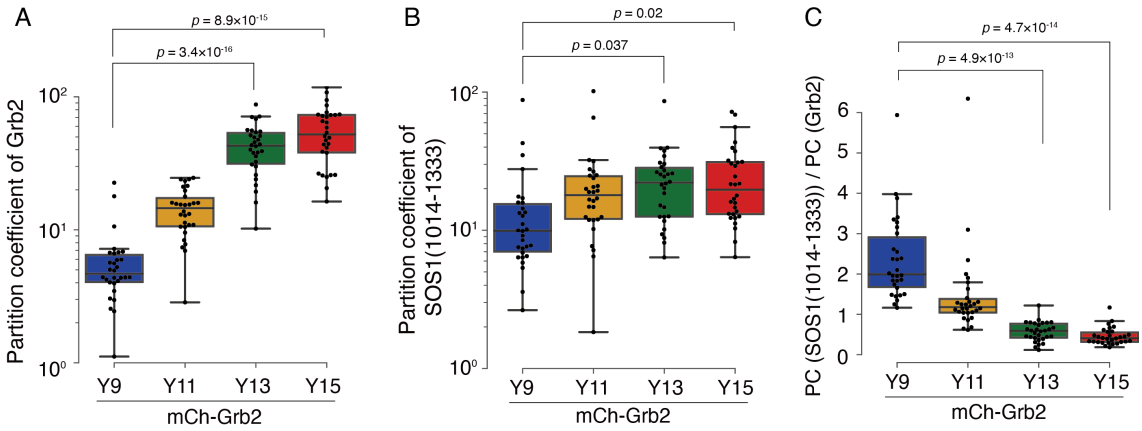

Fig. S11 Partition coefficients of bait Grb2 and prey SOS1(1014-1333). (A-B) Box plots of the PCs of (A) Yn-mCh-Grb2 and (B) mTagBFP2-SOS1(1014-1333). The PC values of prey SOS1(1014-1333) were high even in the case of Y9 tag; there were no significant difference among Y11–Y15 (p = 0.67 (Y11 vs Y13), 0.46 (Y11 vs Y15), 0.71 (Y13 vsY15)). (C) A box plot of PC ratio (SOS1(1014-1333) versus Grb2). At the Y9 tag, the PC ratio shows high value (2.4 $\pm$ 1.0). These boxplots are presented with the elements: center line, median; box limits, Q1 and Q3; whiskers, 1.5× interquartile range; points, outliers. p-values; two-tailed paired t-tests. (n = 30 cells over three biologically independent experiments).

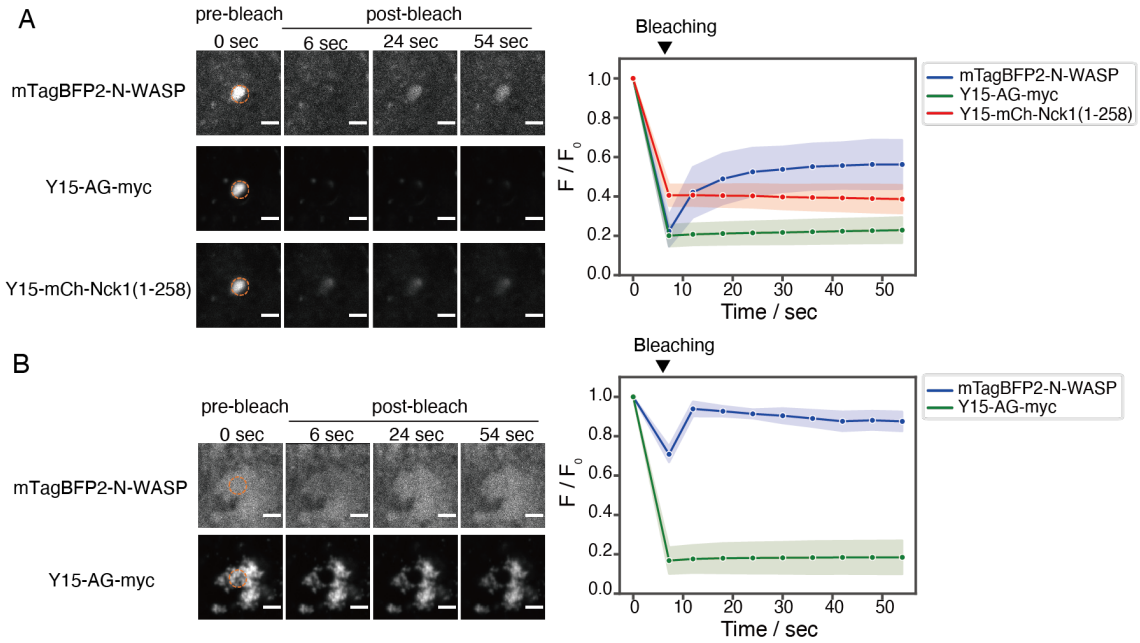

Fig. S12 FRAP measurements of mTagBFP2-N-WASP inside the granules. (A) Time-lapse images and FRAP recovery profile in the presence of Nck1(1-258). The gradual recovery of fluorescence on the whole bleached granule was observed. The data points are presented as mean values with +/- S.D. as translucent error bands (n = 13 cells). (B) Time-lapse images and the profile in the absence of Yn-mCh-Nck1(1-258). The diffusion rate of mTagBFP2-N-WASP was too fast to be bleached, indicating that the prey protein was not physically captured into the Y15-AG-myc scaffold. The data points are presented as mean values with +/- S.D. as translucent error bands (n = 5 cells).

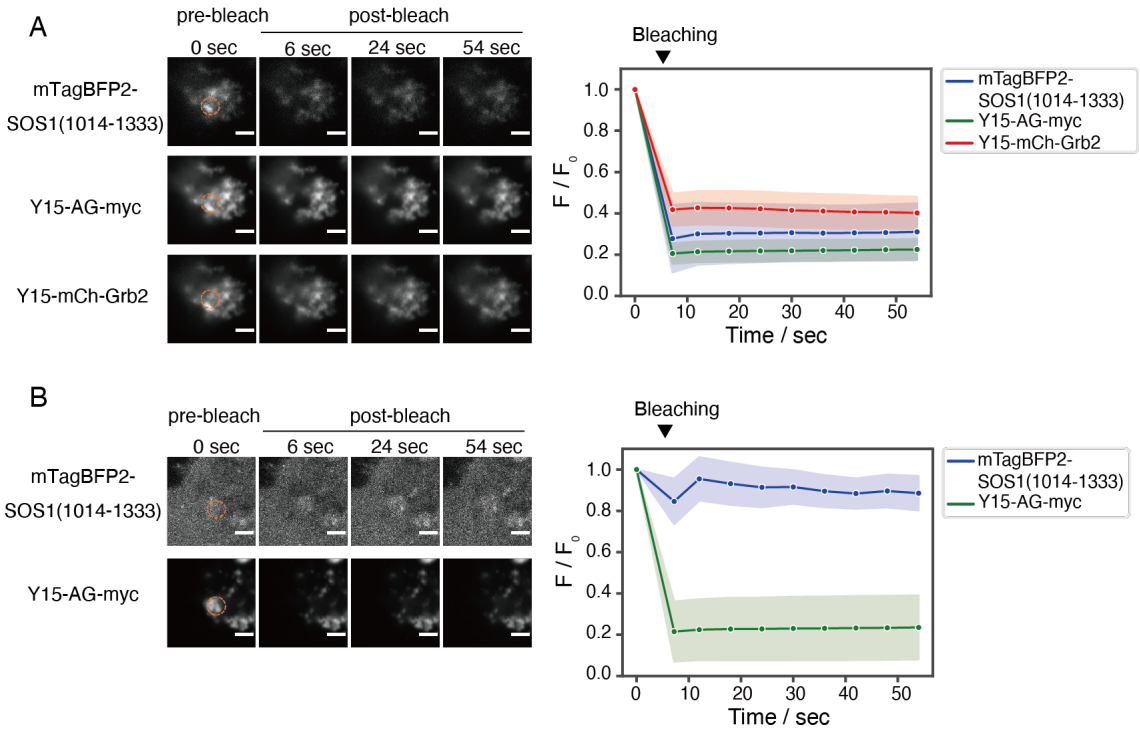

Fig. S13 FRAP measurements of mTagBFP2-SOS1(1014-1333) inside the granules. (A) Time-lapse images and the profile in the presence of Grb2. Any fluorescence recovery was not observed. The data points are presented as mean values with +/- S.D. as translucent error bands (n = 13 cells). (B) Time-lapse images and the profile in the absence of Yn-mCh-Grb2. The diffusion rate of mTagBFP2-SOS1(1014-1333) was too fast to be bleached, indicating that the prey protein was not physically captured into the Y15-AG-myc scaffold. The data points are presented as mean values with +/- S.D. as translucent error bands (n = 5 cells).

Reference

1 T. Miki, T. Nakai, M. Hashimoto, K. Kajiwara, H. Tsutsumi and H. Mihara, *Nat. Commun.*, 2021, **12**, 3412.

2 R. W. Strange, S. Antonyuk, M. A. Hough, P. A. Doucette, J. A. Rodriguez, P. J. Hart, L. J. Hayward, J. S. Valentine and S. S. Hasnain, *J. Mol. Biol.*, 2003, **328**, 877–891.

3 C. D. Putnam, A. S. Arvai, Y. Bourne and J. A. Tainer, *J. Mol. Biol.*, 2000, **296**, 295–309.

4 P. Press and D. O. I. Rev, *J. Biol. Chem.*, 2019, **294**, 2085–2097.

5 C. Scheufler, A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F. U. Hartl and I. Moarefi, *Cell*, 2000, **101**, 199–210.