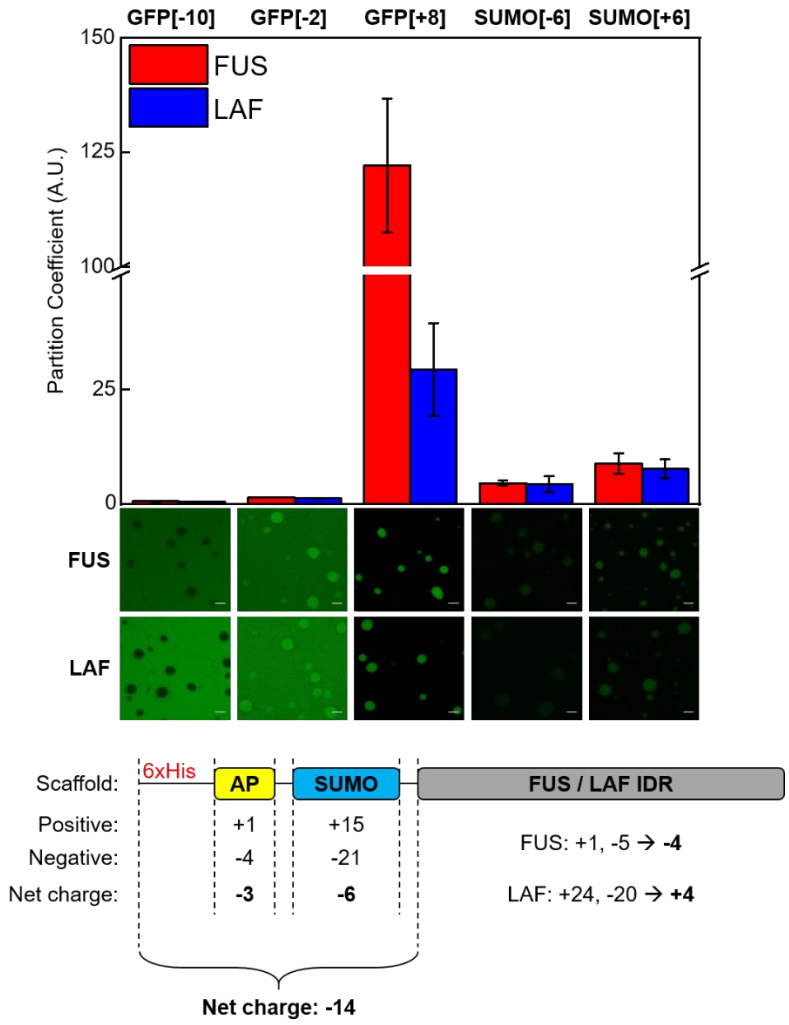


Figure S1 Recruitment degrees of GFP and SUMO charge variants into FUS (red) and LAF (blue) condensates with a biotinylated SUMO (-14 net charge) fusion. Representative confocal images of client recruitment inside condensates are shown below the graph. Scale bars: 10 μm. Error bars: 1 s.d. (n = 100 from three independent experiments). Schematic diagram for biotin-SUMO fused IDR constructs with net charges for each module is shown in the bottom.

Note: The AP peptide for biotin tagging has a -3 net charge, and original SUMO has a -6 net charge. Linkers and between a 6His tag, AP and SUMO have a total -5 net charge to make a final -14 net charge.

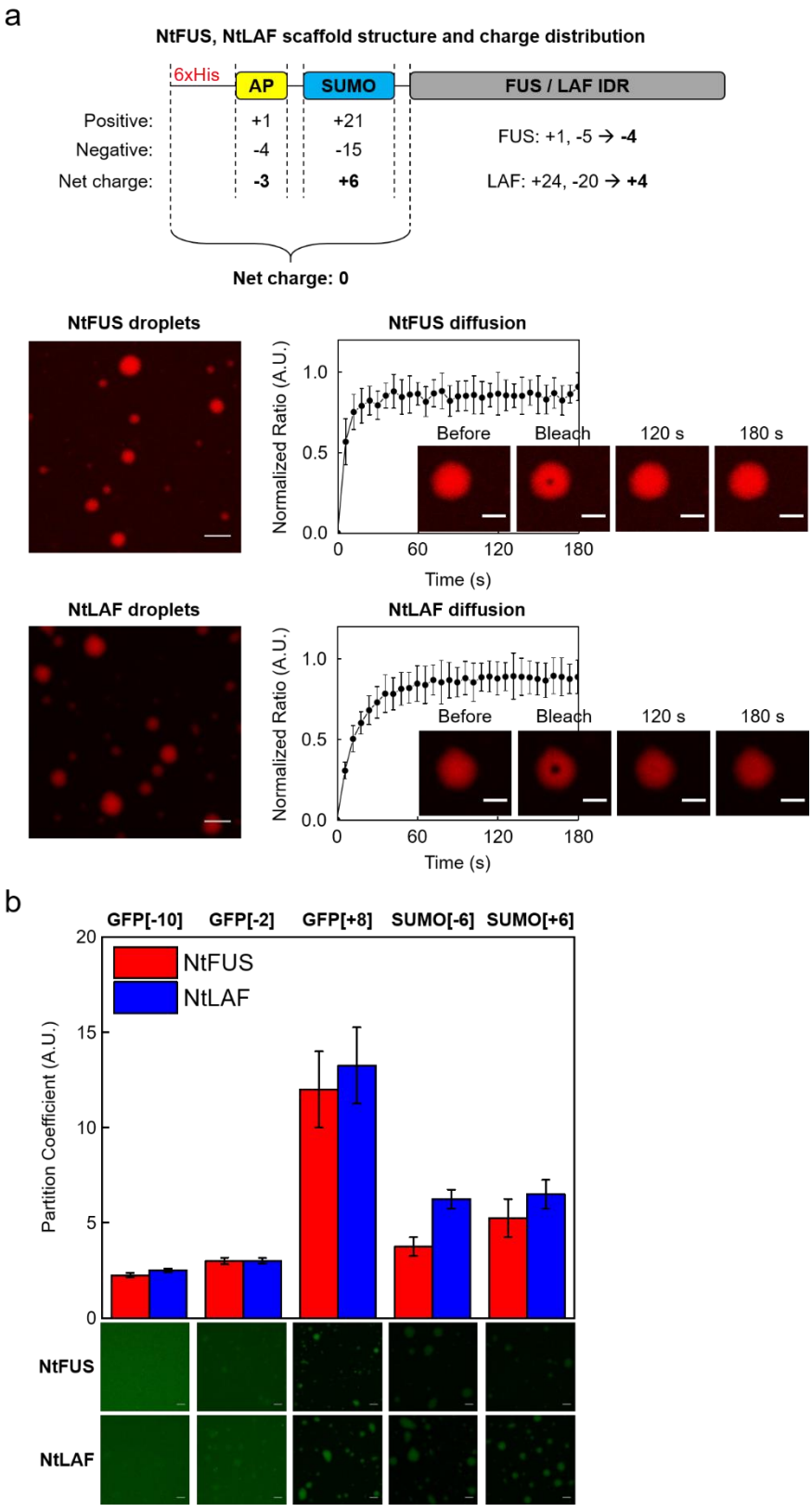
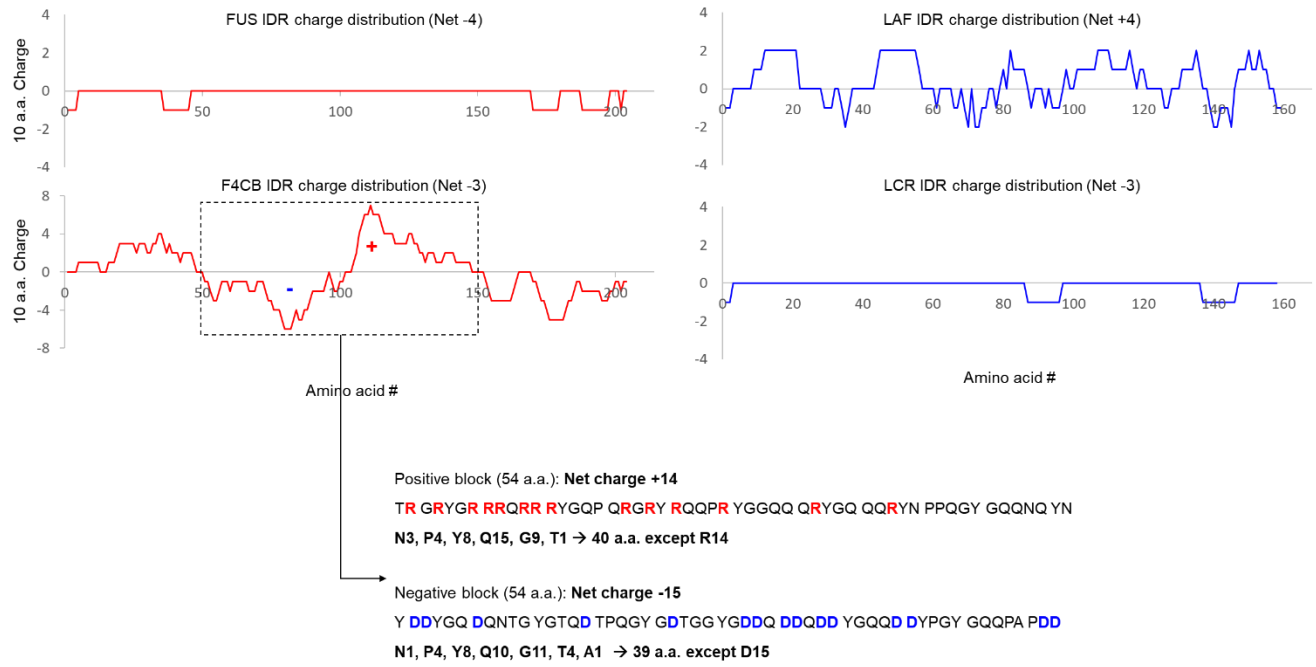


Figure S2 Condensate formation of IDRs with a neutral biotin-SUMO (NtFUS and NtLAF). (a) Phase separation and inside diffusion tests of NtFUS and NtLAF. Schematic diagram for neutralized biotin-SUMO fused IDR constructs with net charges for each module is shown at the top. Confocal images of condensates and fluorescence recovery after photobleaching (FRAP) experiments are shown. Scale

bars: 10 μm (left image), 5 μm (right, diffusion images). Relative fluorescence signal recovery profiles are also shown. Error bars: 1 s.d. (n = 15 from three independent experiments.) (b) Recruitment degrees of GFP and SUMO charge variants into NtFUS (red) and NtLAF (blue) condensates. Representative confocal images of client recruitment inside condensates are shown below the graph. Scale bars: 10 μm. Error bars: 1 s.d. (n = 100 from three independent experiments).

Note: The preferred recruitment of GFP[+8] and SUMO[+6] over negative proteins was significantly reduced for neutral NtFUS and NtLAF condensates, compared to FUS and LAF condensates (Figure S1).



Positive block (54 a.a.): **Net charge +14**

TR GRYGR RRQRR RYGQP QRGRY RQQPR YGGQQ QRYGQ QQRYN PPQGY GQQNQ YN

N3, P4, Y8, Q15, G9, T1 → 40 a.a. except R14

Negative block (54 a.a.): **Net charge -15**

Y DDYGQ DQNTG YGTQD TPQGY GDTGG YGDDQ DDQDD YGQQD DYPGY GQQPA PDD

N1, P4, Y8, Q10, G11, T4, A1 → 39 a.a. except D15

Figure S3 Charge distribution plots of IDR charge variants (FUS, LAF, F4CB, and LCR). Charge values on y axis are calculated from net charges of 10 amino acids windows. Negative and positive blocks in the middle of F4CB (dotted box) are indicated with their sequence information. These blocks are used to generate charge-biased clients (2NB~2PB).

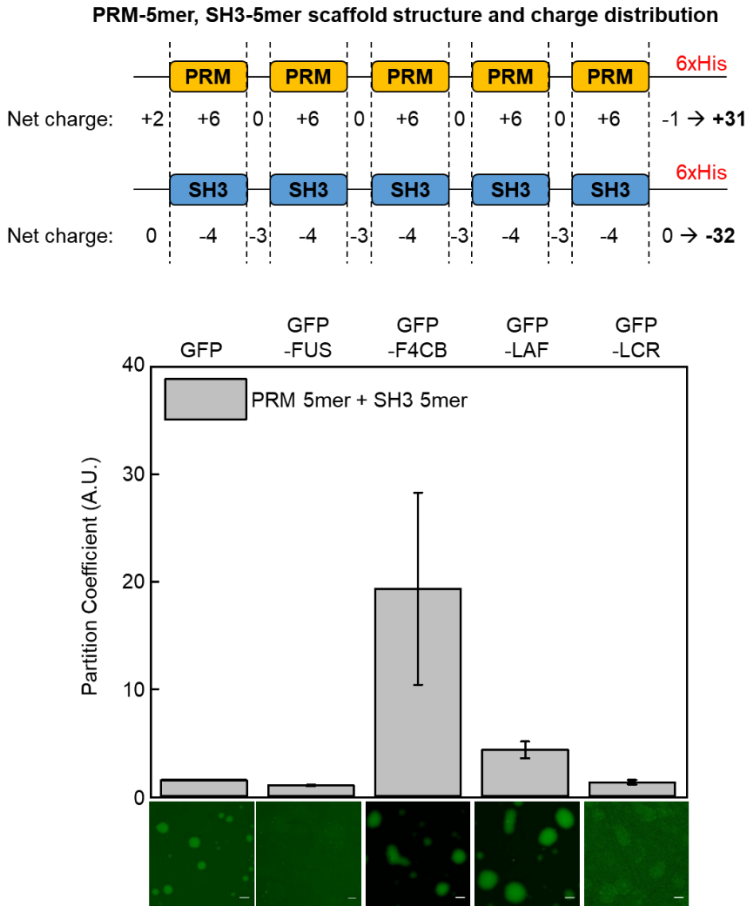


Figure S4 Recruitment degrees of GFP-IDR clients into PRM-SH3 condensates. Representative confocal images of client recruitment inside condensates are shown below the graph. Scale bars: 10 μm. Error bars: 1 s.d. (n = 100 from three independent experiments). Schematic diagram for PRM and SH3 pentamer constructs with net charges for each module is shown at the top.

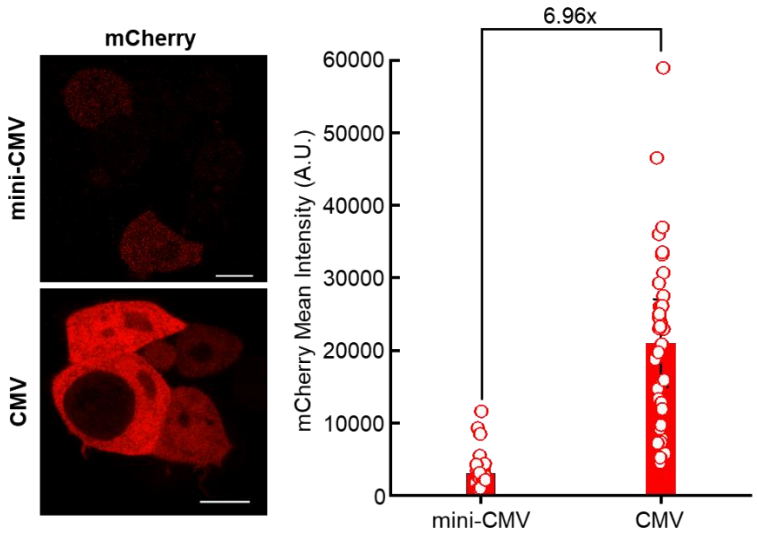


Figure S5 Relative expression levels of mCherry with the mini-CMV (top) or CMV (bottom) promoter in cells. Scale bars: 10 μm. Error bars: 1 s.d. (n = 37 cells)

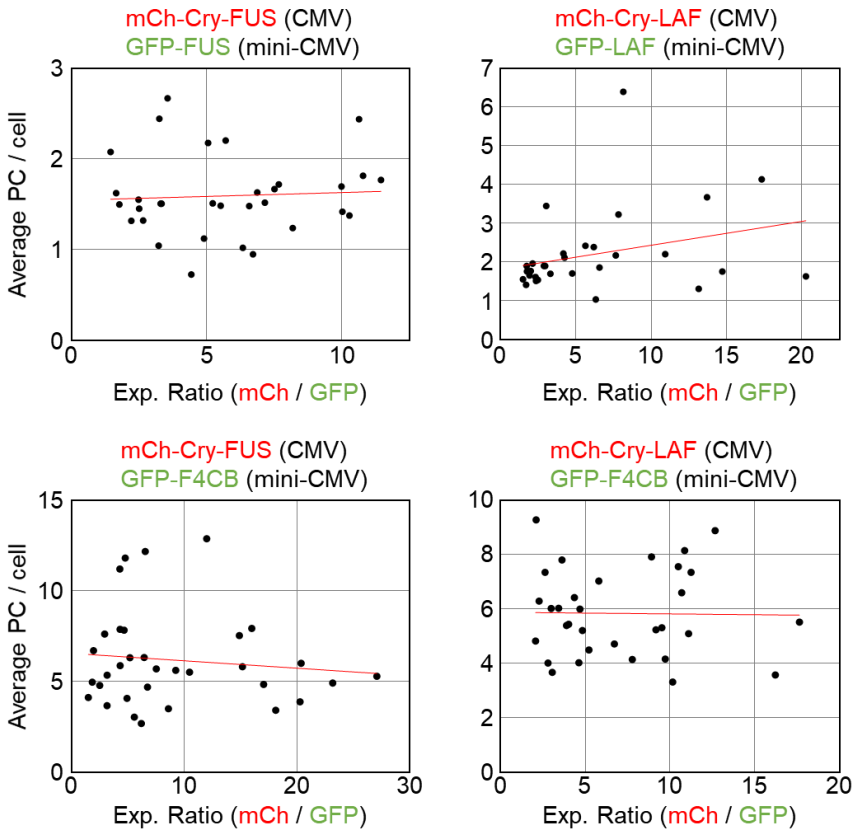


Figure S6 Cell-to-cell recruitment degree variations with regard to relative scaffold (mCh)-client (GFP) expression ratios.

Note: Both relative mCh/GFP signals and PC values are highly varied but show no clear correlation.

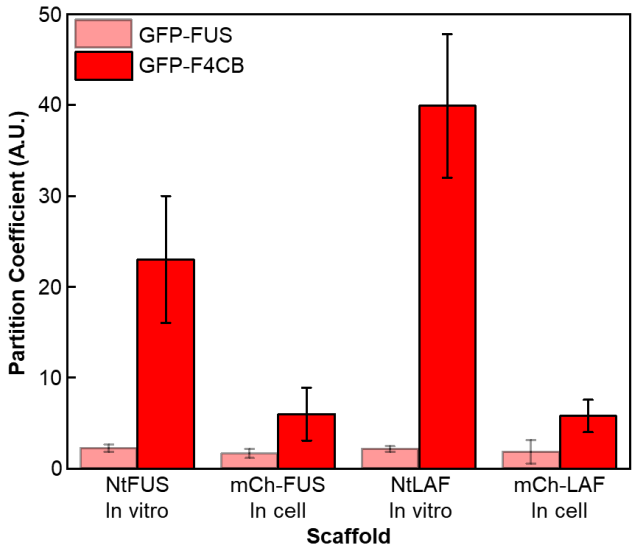


Figure S7 Recruitment degrees of GFP-FUS and GFP-F4CB into FUS and LAF condensates *in vitro* (Scaffold: NtFUS and NtLAF) and in cells (Scaffold: mCh-FUS and mCh-LAF). All PC data are identical to those in Figure 1.

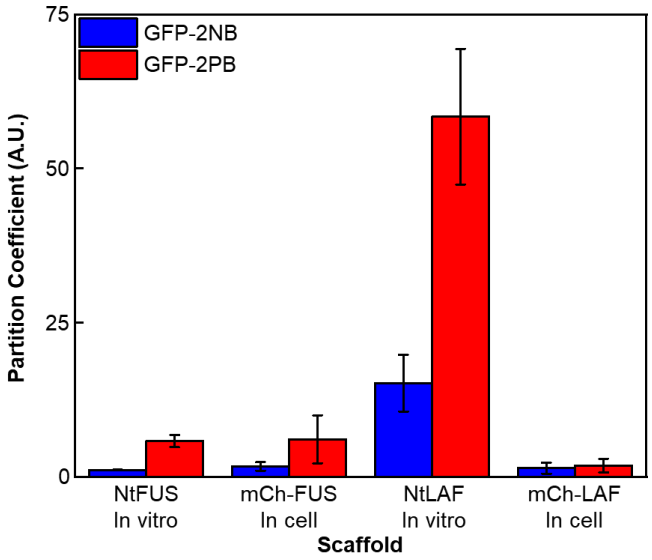


Figure S8 Recruitment degrees of GFP-2NB and GFP-2PB into FUS and LAF condensates *in vitro* (Scaffold: NtFUS and NtLAF) and in cells (Scaffold: mCh-FUS and mCh-LAF). All PC data are identical to those in Figure 2.

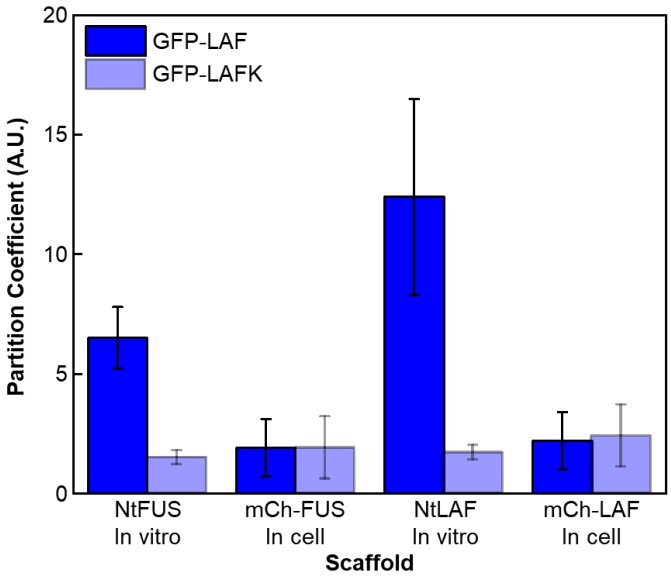


Figure S9 Recruitment degrees of GFP-LAF and GFP-LAFK into FUS and LAF condensates *in vitro* (5% PEG) and in cells. All PC data are identical to those in Figure 3.

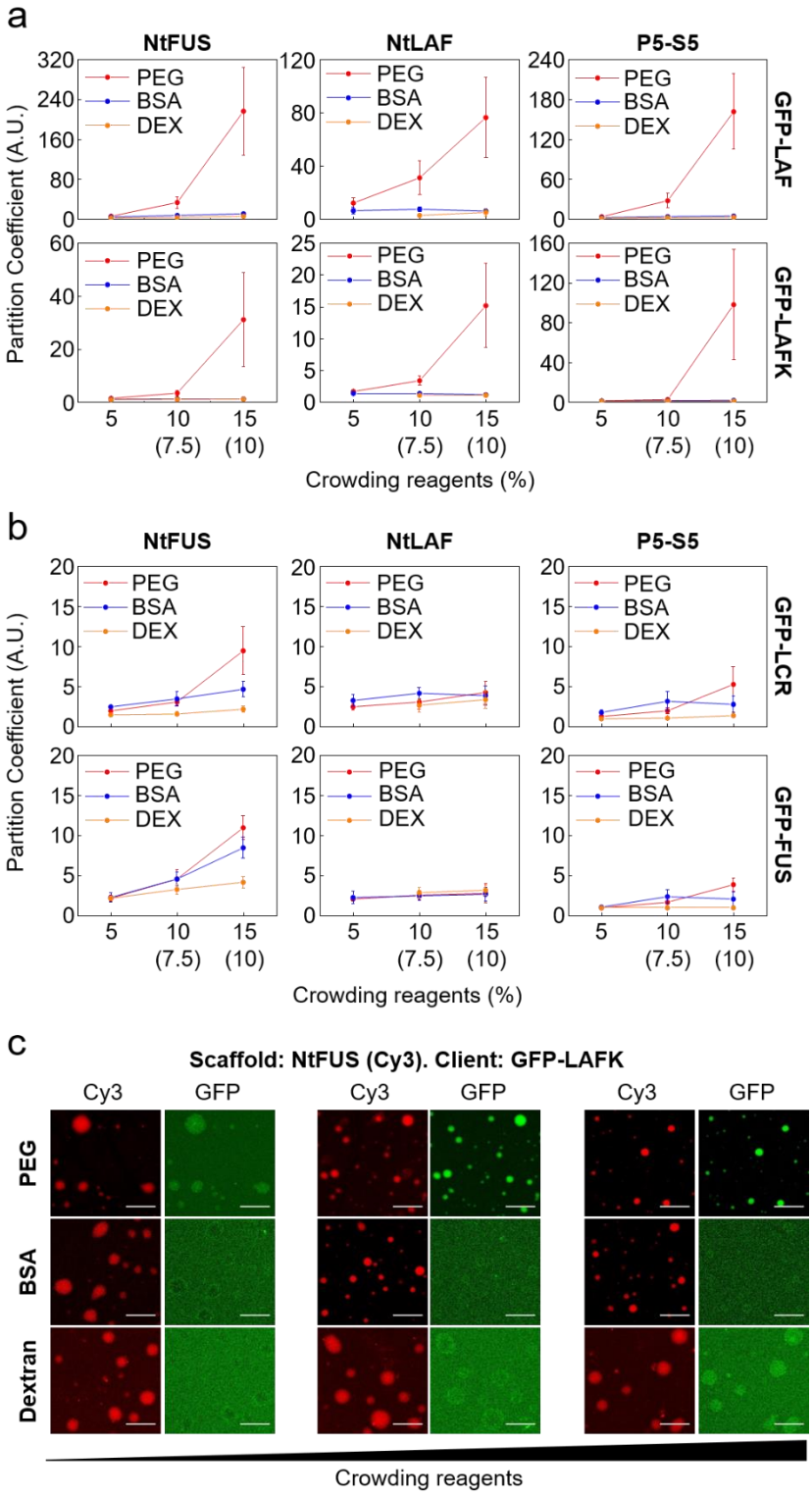


Figure S10 Recruitment PC changes of (a, c) Arg-to-Lys mutated (LAF and LAFK) and (b) charge-deficient FUS and LCR clients with increasing concentrations of three crowding reagents. BSA was used with 2% PEG, and dextran (DEX) was used at 5%, 7.5%, 10% instead of 5%, 10%, 15% for PEG and BSA. Error bars: 1 s.d. (n = 100 from three independent experiments).

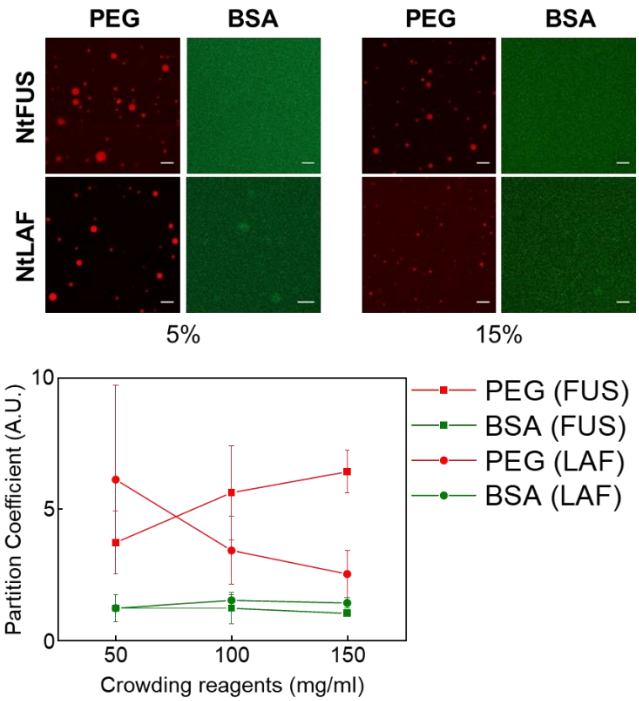


Figure S11 Localization of PEG and BSA inside IDR condensates under varying crowding conditions. Fluorescent dye-labeled PEG or BSA was incubated with IDR condensates with indicated concentrations of PEG and BSA crowding reagents. Scale bars: 10 μm. Error bars: 1 s.d. (n=100 from three independent experiments).

Note: In/out PCs of dye-PEG is 2.5~6.4, indicating higher PEG concentrations inside condensates than outside. On the other hand, in/out PCs of dye-BSA is 1~1.5, indicating that BSA proteins are mostly evenly distributed inside and outside condensates.

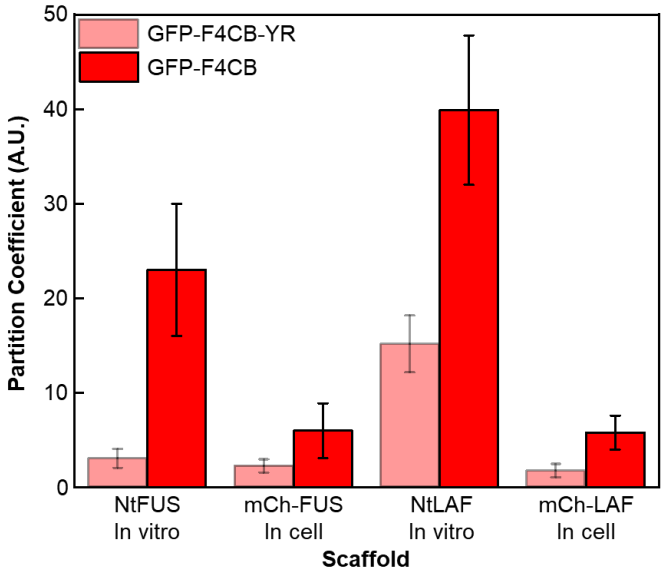


Figure S12 Recruitment degrees of GFP-F4CB-YR and GFP-F4CB into FUS and LAF condensates *in vitro* (5% PEG) and in cells. All PC data are identical to those in Figure 1 and Figure 4.

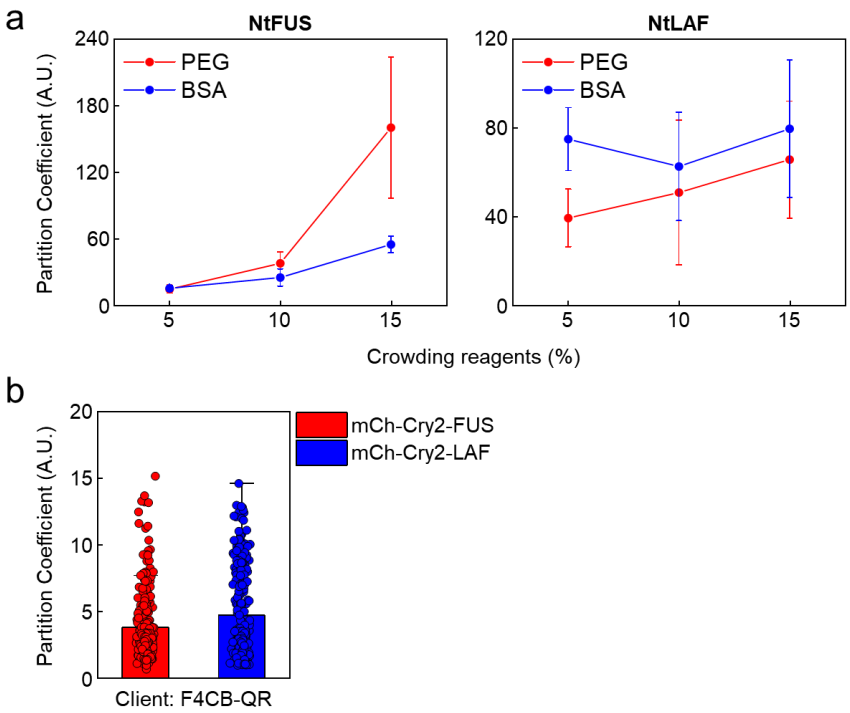


Figure S13 Recruitments of Gln-removed F4CB-QR clients. (a) Recruitment PCs of GFP-F4CB-QR into IDR condensates *in vitro* with varying concentrations of crowding reagent PEG and BSA. Error bars: 1 s.d. (n = 100 from three independent experiments). (b) F4CB-QR recruitments in cells. Error bars: 1 s.d. ($n \geq 30$ cells)

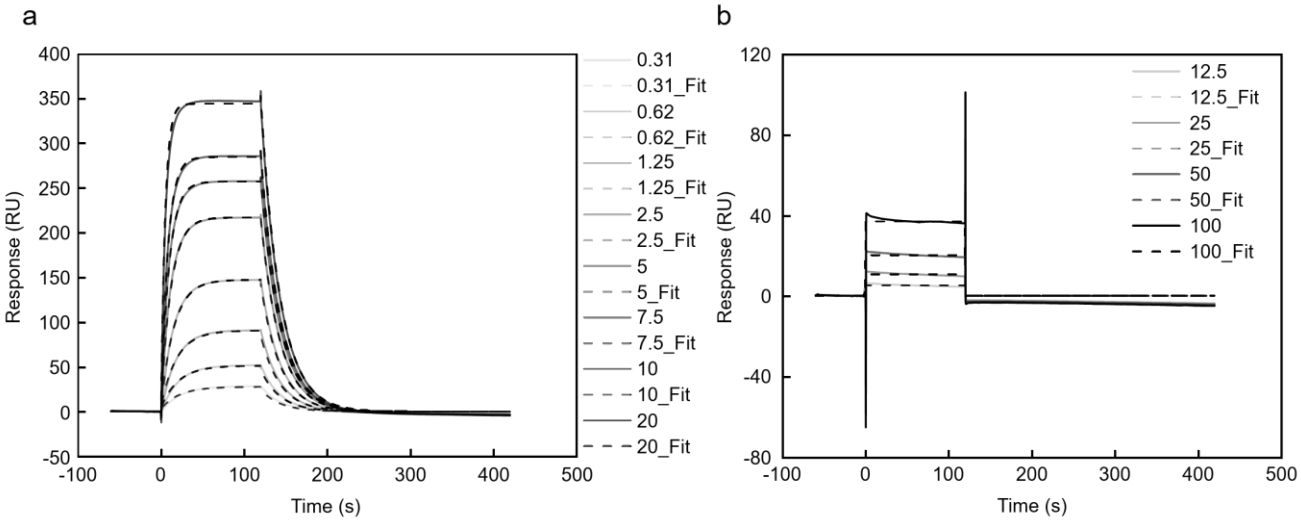


Figure S14 Surface plasmon resonance (SPR) analysis of binding affinities between D7N (a) and D7Ns (b) with SC. Observed SPR binding responses (solid lines) and fitting kinetic data (dotted lines) are shown together. Introduced SC concentrations (μM) are indicated.

Note: The calculated (fitted) Kd value of a D7N and SC interaction is 5 μM (a), whereas binding between shortened D7Ns and SC is not observed even with over 100 μM SC (b).

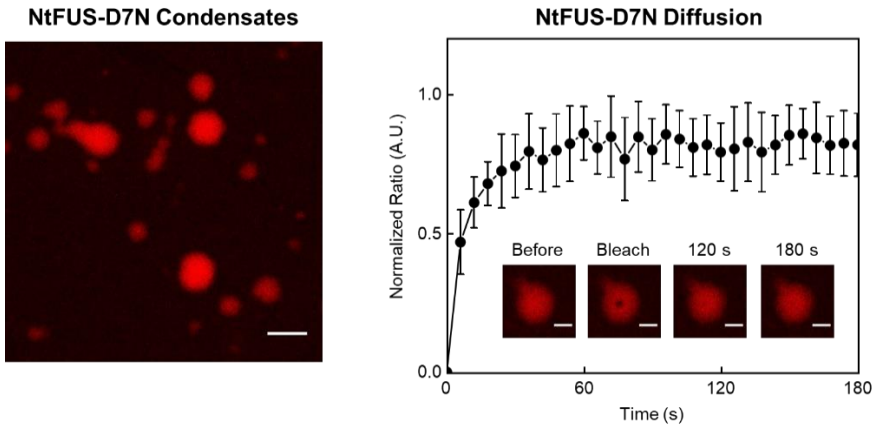


Figure S15 Phase separation and inside diffusion tests of NtFUS-D7N. Confocal images of condensates and FRAP experiments are shown. Scale bars: 10 μm (left image), 5 μm (right, diffusion images). Relative fluorescence signal recovery profiles are also shown. Error bars: 1 s.d. (n = 15 from three independent experiments.)

Note: The NtLAF with D7N scaffold was not expressed enough to proceed phase separation experiments *in vitro*.

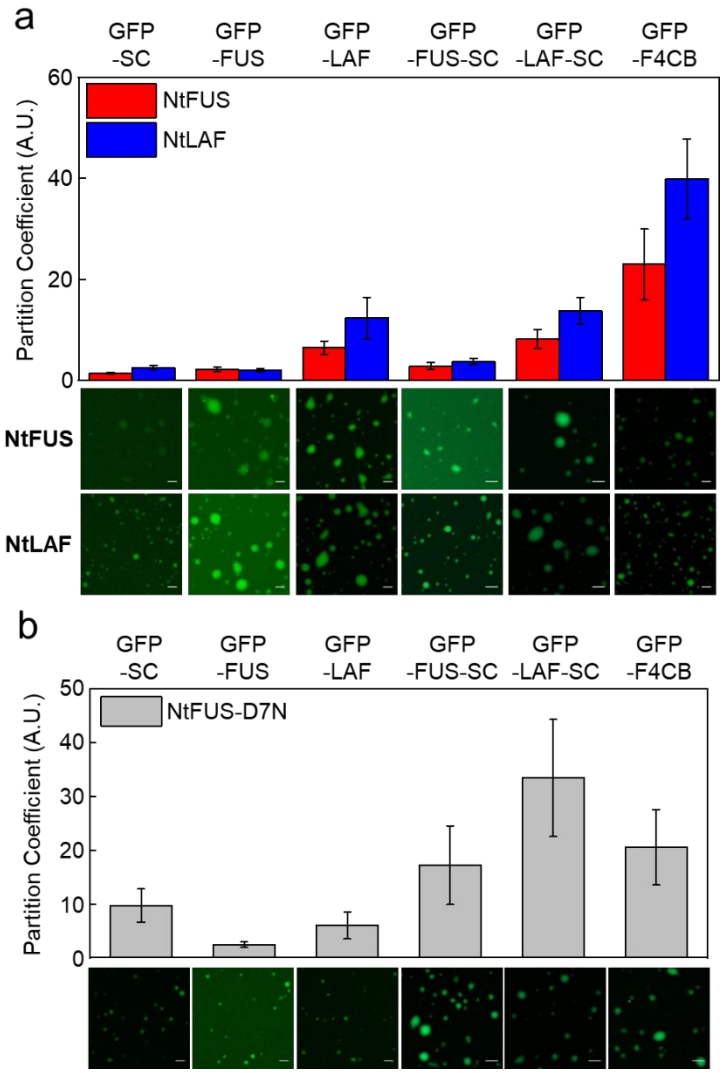


Figure S16 Recruitment PCs of client proteins with SC or IDR. (a) Recruitment PCs of client proteins with SC or IDR into IDR condensates without D7N (5% PEG). (b) Recruitment PCs of client proteins with SC or IDR into NtFUS-D7N condensates with 5% BSA and 2% PEG. Scale bars: 10 μm. Error bars = 1 s.d. (n = 100 from three independent experiments).

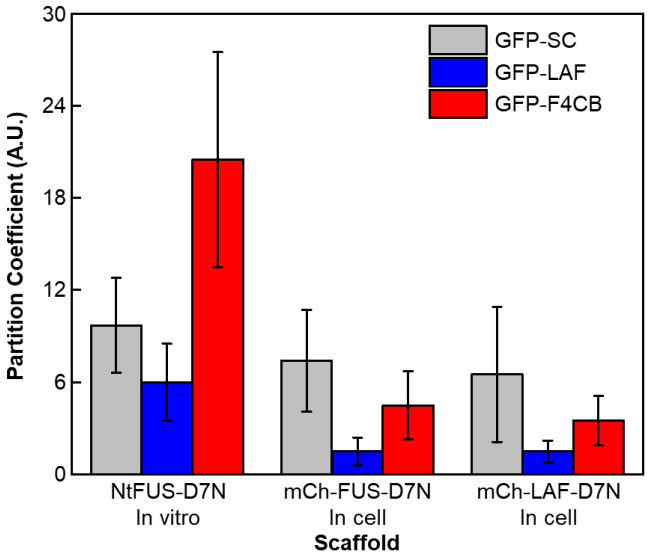


Figure S17 Recruitment degrees of GFP-SC, GFP-LAF, and GFP-F4CB into D7N-fused condensates *in vitro* (5% PEG) and in cells. All PC data are identical to those in Figure 5.

Protein sequences

Scaffolds (Underline: IDR, Red: 6xHis tag, Blue: TEV protease site, Cyan: AP tag, Gray: SUMO [+6])

FUS (Negative scaffold. Gray: SUMO [-6]**)**

MSYYHHHHHHDYDIPTTENLYFQGAMEGLNDIFEAQKIEWHEMSDSEVNQEAKPEVKPEVKPETHIN
LKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHRE
QIGGATYEFASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSSYG
QSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQPQS
GSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSG
GGYGNQDQSGGGGSGGYGQQDRG

LAF (Negative scaffold. Gray: SUMO [-6]**)**

MSYYHHHHHHDYDIPTTENLYFQGAMEGLNDIFEAQKIEWHEMSDSEVNQEAKPEVKPEVKPETHIN
LKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHRE
QIGGATYEFMESNQSNNGGSGNAALNRGGRYVPPHLRGGDGGAAAAASAGGDDRRGGAGGGGYR
RGGGNSGGGGGGGYDRGYNDNRDDRDNRGGSGGYGRDRNYEDRGYNGGGGGGGNRGYNNNRG
GGGGGYNRQDRGDGGSSNFSRGGYNNRDEGSDNRGSGRSYNNDRRDNGGDG

NtFUS

MSYYHHHHHHDYDIPTTENLYFQGAMGGLNDIFEAQKIEWHEMSKSKVNQEAKPEVKPEVKPKTHI
NLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPKDLDMKDNDIIEAH
RKQIGGATYGTASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSS
YGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQP
QSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGS
GGGYGNQDQSGGGGSGGYGQQDRG

NtFUS-D7N

MSYYHHHHHHDYDIPTTENLYFQGAMGGLNDIFEAQKIEWHEMSKSKVNQEAKPEVKPEVKPKTHI
NLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPKDLDMKDNDIIEAH
RKQIGGATYGTASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSS
YGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQP
QSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGS
GGGYGNQDQSGGGGSGGYGQQDRGAHIVMVNAYKPTK

NtFUS-D7Ns

MSYYHHHHHHDYDIPTTENLYFQGAMGGLNDIFEAQKIEWHEMSKSKVNQEAKPEVKPEVKPKTHI
NLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPKDLDMKDNDIIEAH
RKQIGGATYGTASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSS
YGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQP
QSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGS
GGGYGNQDQSGGGGSGGYGQQDRGAHIVMVNA

NtLAF

MSYYHHHHHHDYDIPTTENLYFQGAMGGLNDIFEAQKIEWHEMSKSKVNQEAKPEVKPEVKPKTHI
NLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPKDLDMKDNDIIEAH
RKQIGGATYGTMESNQSNNGGSGNAALNRGGRYVPPHLRGGDGGAAAAASAGGDDRRGGAGGGG
YRRGGGNSGGGGGGGYDRGYNDNRDDRDNRGGSGGYGRDRNYEDRGYNGGGGGGGNRGYNNN
RGGGGGGYNRQDRGDGGSSNFSRGGYNNRDEGSDNRGSGRSYNNDRRDNGGDG

PRM-5mer (PRM: Underline)

MASMTGGQQMGRGSEFKGGSWGRSKKKKTAPTPPKRSGGSGGSGGSGGSKKKKTAPTPPKRSGGS
GGSGGSGGSKKKKTAPTPPKRSGGSGGSGGSGGSKKKKTAPTPPKRSGGSGGSGGSGGSKKKKTAPT
PPKRSGGSGSGVDIEGRHHHHHH

SH3-5mer (SH3: Underline)

MASMTGGQQMGRGSGVDDLNMPAYVKFNYMAEREDELSLIKGTKVIVMEKSSDGWWRGSYNGQV
GWFPSNYVTEEGDSPLLDASGAGGSEGGGSEGGTSGATDLNMPAYVKFNYMAEREDELSLIKGTKVI
VMEKSSDGWWRGSYNGQVGWFPSNYVTEEGDSPLLDASGAGGSEGGGSEGGTSGATDLNMPAYVK
FNYMAEREDELSLIKGTKVIVMEKSSDGWWRGSYNGQVGWFPSNYVTEEGDSPLLDASGAGGSEG
GGSEGGTSGATDLNMPAYVKFNYMAEREDELSLIKGTKVIVMEKSSDGWWRGSYNGQVGWFPSNY
VTEEGDSPLLDASGAGGSEGGGSEGGTSGATDLNMPAYVKFNYMAEREDELSLIKGTKVIVMEKSSD
GWWRGSYNGQVGWFPSNYVTEEGDSPLIEGRHHHHHH

Clients (Underline: IDR, Red: 6xHis tag, Green: GFP[-2], Gray: SpyCatcher)

GFP [-10]

HMKGEELFTGVVPILVELDGDVNGHEFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHDVYITADKQENGIKAEFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITLEHHHHHH

GFP [-2]

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITLEHHHHHH

GFP [+8]

HMKGERLFTGVVPILVELDGDVNGHKFSVRGKGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPKGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIKLKGTDFKEDG
NILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGRGPVLLPDNHYL
STQTVLSKDPNEKRDHMVLHEYVNAAGITLEHHHHHH

SUMO [-6]

HMMSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFL
YDGIRIQADQTPEDLDMEDNDIIEAHREQIGGATYLEHHHHHH

SUMO [+6]

HMMSKSKVNQEAKPEVKPEVKPKTHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRF
LYDGIRIQADQTPKDLDMKDNDIIEAHRKQIGGATYLEHHHHHH

GFP-FUS

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS

TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQ
SYSGYSQSTDTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQ
PAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGG
GGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRGLEHHHHHH

GFP-F4CB

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMARNDYTQQATQRYGAYPTQPGQGYRQQRRQPYGQ
QRYRGYRQRTDTRGYGQRRYDDYGQDQNTGYGTQDTPQGYGDTGGYGDDQDDQDDYGQQDDYP
GYGQQPAPDDTRGRYGRRRQRRRYGQPQRGRYRQQPRYGGQQQRYGQQQRYNPPQGYGQQNQYN
DDDGGGGGGGGGGNYGQDQDDMDDGGGDGGGYGNQDQDGGGGDGGYGQQDRGLEHHHHHH

GFP-F4CB-QR

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMARNDYTSSATSRYGAYPTNPGQGYRNQRRQPYGN
QRYRGYRNRTDTRGYGNRRYDDYGNDQNTGYGTNDTPNGYGDTGGYGDDNDDSDDYGNNDDYP
GYGNQPAPDDTRGRYGRRRNRRRYGNPQRGRYRNQPRYGGNQSRYGNQSRYNPPNGYGQNNQYN
DDDGGGGGGGGGGNYGSDSDDMDDGGGDGGGYGNSDSDGGGGDGGYGNQDRGLEHHHHHH

GFP-F4CB-YR

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMARNDNTQQATQRYGASPTQPGQGYRQQRRQPSGQ
QRSRGYRQRTDTRGNGQRRSDDYGQDQNTGYGTQDTPQGNGDTGGYGDDQDDQDDSGQQDDSPG
YGQQPAPDDTRGRSGRRRQRRRNGQPQRGRSRQQPRYGGQQQRYGQQQRNNPPQGYGQQNQSND
DDGGGGGGGGGGNNGQDQDDMDDGGGDGGGSGNQDQDGGGGDGGYGQQDRGLEHHHHHH

GFP-2NB

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFYDDYGQDQNTGYGTQDTPQGYGDTGGYGDDQDDQ
DDYGQQDDYPGYGQQPAPDDAAAYDDYGQDQNTGYGTQDTPQGYGDTGGYGDDQDDQDDYGQQ
DDYPGYGQQPAPDDLEHHHHHH

GFP-1NB

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFYDDYGQDQNTGYGTQDTPQGYGDTGGYGDDQDDQ
DDYGQQDDYPGYGQQPAPDDLEHHHHHH

GFP-1PB

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFTRGRYGRRRQRRRYGQPQRGRYRQQPRYGGQQQRY
GQQQRYNPPQGYGQQNQYNLEHHHHHH

GFP-2PB

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFTRGRYGRRRQRRRYGQPQRGRYRQQPRYGGQQQRY
GQQQRYNPPQGYGQQNQYNAAATRGRYGRRRQRRRYGQPQRGRYRQQPRYGGQQQRYGQQQRYN
PPQGYGQQNQYNLEHHHHHH

GFP-2PBK

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFTKGKYGKKKQKKKYGQPQKGKYKQQPKYGGQQQK
YGQQQKYNPPQGYGQQNQYNAAATKGKYGKKKQKKKYGQPQKGKYKQQPKYGGQQQKYGQQQ
KYNPPQGYGQQNQYNLEHHHHHH

GFP-LAF

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMESNQSNNGGSGNAALNRGGRYVPPHLRGGDGGAA
AAASAGGDDRRGGAGGGGYRRGGGNSGGGGGGGYDRGYNDNRDDRDNRGGSGGYGRDRNYEDR
GYNGGGGGGGNRGYNNNRGGGGGGYNRQDRGDGGSSNFSRGGYNNRDEGSDNRGSGRSYNNDR
RDNGGDGLEHHHHHH

GFP-LAFK

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMESNQSNNGGSGNAALNKGGKYVPPHLKGGDGGAA
AAASAGGDDKKGGAGGGGYKKGGGNSGGGGGGGYDKGYNDNKDDKDNKGGSGGYGKDKNYED
KGYNGGGGGGGNKGYNNNKGGGGGGYNKQDKGDGGSSNFSKGGYNNKDEGSDNKGSGKSYNND
KKDNGGDGLEHHHHHH

GFP-LCR

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMESNQSNNGGSGNAALNYGGSYVPPHLYGGYGGAA
AAASAGGSSSYGGAGGGGYSSGGGNSGGGGGGGYSSGYNSNSSSYSNSGGSGGYGSSSNYESSGYN
GGGGGGGNSGYNNNSGGGGGGYNSQSSGYGGSSNFSSGGYNNSSEGSYNSGSGSSYNNSSSYNGGS
GLEHHHHHH

GFP-SpyCatcher

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDE
DGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTV
NGKATKGDAHILEHHHHHH

GFP-FUS-SpyCatcher

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQ
SYSGYSQSTDTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGGYGSSQSSQSSYGQQSSYPGYGQQ
PAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQYNSSSGGGGG
GGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRGKLGAMVDTLSGLSSEQGQSG
DMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDG
YEVATAITFTVNEQGQVTVNGKATKGDAHILEHHHHHH

GFP-LAF-SpyCatcher

HMKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGV
QCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGN
ILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLS
TQTVLSKDPNEKRDHMVLHEYVNAAGITEFMESNQSNNGGSGNAALNRGGRYVPPHLRGGDGGAA
AAASAGGDDRRGGAGGGGYRRGGGNSGGGGGGGYDRGYNDNRDDRDNRGGSGGYGRDRNYEDR
GYNGGGGGGGNRGYNNNRGGGGGGYNRQDRGDGGSSNFSRGGYNNRDEGSDNRGSGRSYNNDR
RDNGGDGKLGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTIS
TWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHILEHHHHHH

Experimental methods

Protein preparation. NtFUS, NtFUS-D7N, NtFUS-D7Ns and NtLAF genes were cloned into the pProExHTα expression vector (Invitrogen), and the plasmids were transformed in AVB101 (Avidity), an *E.coli* B strain (hsdR, lon11, SulA1) containing a pACYC184 plasmid, which produces biotin ligase BirA. The transformed cells were grown at 37 °C until OD600 reaches 0.8, and proteins were induced with 0.05 mM biotin and 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG), followed by incubation at 37 °C for 6 h. After IPTG induction, cells were harvested by centrifugation at 6000 rpm for 5 min. The cells were lysed by sonication in the equilibration buffer (500 mM NaCl, 50 mM Tris with pH 8.0, 10 mM Imidazole, 10% Glycerol). Lysed cells were then centrifuged at 12000 rpm for 15 min at 4 °C, and the supernatants were purified by Ni-IDA columns (BioProgen, Daejeon, South Korea). Purified proteins were added by final 5 mM EDTA to remove nickel ions, and dialyzed into the storage buffer (100 mM NaCl, 50 mM Tris-HCl with pH 8.0, 10% Glycerol) at 25 °C, and centrifugally concentrated by Vivaspin 20 columns with a 10 kDa molecular weight cutoff (GE Healthcare) until the volume became less than 1 mL. Concentrated proteins were incubated at 60 °C with a heat block (ALB6400, FINEPCR Co.) for 20 min to remove impurities by selective aggregation of non-specific proteins. Aggregates were centrifuged, and further purified IDR proteins were finally filtrated through a 0.2 μm membrane filter (Advantec, DISMIC-13 CP). Final protein concentrations were measured by A280, and the proteins were centrifugally concentrated again until concentrations reach the range 100~150 μM. Finally protein samples were distributed to 50~70 μL aliquots, flash-frozen by liquid nitrogen and stored in -80 °C.

Free GFP, SUMO, GFP-tagged clients and PTM-5mer, SH3-5mer genes were cloned into the pET-21a expression vector (EMD Biosciences), and the plasmids were transformed in E.coli BL21 (DE3). The transformed cells were grown at 37 °C until OD600 reaches 0.8, and proteins were induced with 1 mM IPTG and then incubated at 37 °C for 6 h. Proteins were similarly purified by Ni-IDA columns. Final protein concentrations were measured by A280, and protein samples were flash-frozen by liquid nitrogen and stored in -80 °C.

Streptavidin (STA) genes were cloned into the pET-21a expression vector (EMD Biosciences), and the plasmids were transformed in E.coli BL21 (DE3). The transformed cells were grown at 37 °C until OD600 reaches 0.8, and proteins were induced with 1 mM IPTG and then incubated at 37 °C for 4 h. After IPTG induction, cells were harvested and lysed by sonication in a PBS buffer containing 0.1% Triton X-100. Lysed cells were then centrifuged at 12000 rpm for 15 min at 4 °C, and the soluble proteins were discarded. The pellets were completely re-suspended in a PBS buffer containing 0.1% Triton X-100 and centrifuged again at 12000 rpm for 15 min at 4 °C. The washed inclusion bodies

were dissolved in 15 mL of 6 M Guanidine hydrochloride (GuHCl) and 50 mM Tris-HCl (pH 8.0) for overnight at 4 °C. Remained insoluble proteins were then removed by centrifugation at 12000 rpm for 30 min at 4 °C. For refolding, denatured STA was diluted dropwise into 200 mL of rapid-stirring PBS (400 rpm) until solutions become turbid. When solutions became turbid, aggregations were removed by centrifugation at 6000 rpm, 4 °C for 30 min and filtered through a 0.22 μm membrane filter (Stericup® Quick Release, Millipore Express® PLUS). The solutions were maintained at 4 °C for overnight. After overnight, the refolded solutions were added 100 mM NaCl and 50 mM Tris-HCl (pH 8.0) and purified by Ni-IDA columns (BioProgen). The final products were dialyzed into the storage buffer and stored at -20 °C.

In cell studies. mCherry-Cry2-(FUS, LAF, FUS-D7N, LAF-D7N, FUS-D7Ns, LAF-D7Ns) construct genes were cloned into the pcDNA3.1 (+) vector (Invitrogen). GFP-tagged clients were cloned in same vector with a mini-CMV promoter. HEK293T cells with fewer than 20 passages were maintained in Dulbecco's modified Eagles' medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA) at 37 °C under 5% CO_2 in humidified atmosphere. For light-induced phase separation, cells transfected by Cry2-containing scaffolds were irradiated with a 488 nm laser line of LSM800 laser scanning confocal microscope (Carl Zeiss) in time series, 20 cycles with 12 seconds of interval.

Fluorescent dye labelling of IDR proteins. Scaffold proteins (FUS, LAF) were labelled with Cyanine 3 (Cy3) by NHS ester conjugations. Proteins were mixed with dyes in a 1 : 0.5 protein/dye ratio. The mixed solutions were incubated for 30 min at 25 °C with shaking (SLRM-3, SeouLin Bioscience), and dye-conjugated proteins were purified by PD10 desalting columns (Sephadex™ G-25 M, GE Healthcare). Concentrations of labelled proteins were measured by A280.

Liquid droplet formation and observation. Stored scaffold proteins were thawed at 37 °C for 20 min. Scaffold proteins and fluorescent dye-conjugated scaffold proteins were mixed first in a 1.5 mL Eppendorf (ep) tube. The final total concentration of scaffolds was 50 μM, and the dye-tagged scaffolds proportion was 1.5 μM.

STA was mixed in a 1:1 molecule ratio with scaffold proteins to make multivalent IDR clusters, and final 1.5 μM of client protein was added. Lastly, final 50, 100, 150 mg/ml of poly-ethylene glycol (PEG, molecular weight 8000, LPS solution), BSA (Gibco, USA) and dextran (450~550 kDa average molecular mass, Pharmacosmos) were added to induce phase separation. Mixed solutions were maintained for 5 min in tubes. For confocal analysis, 4 μL of solutions with droplets were dropped on a slide glass with hydrophobic surface and covered by a cover glass. For hydrophobic coating of glass

surfaces, slide glasses (Marienfeld) and cover glasses (Duran) were immersed to the piranha acid solution (95% Sulfuric acid 450 mL + 35% Hydrogen peroxide 150 mL) and incubated for 1 h at 60 °C. The glasses were serially washed by distilled water and acetone, and blew by nitrogen gas. The glasses were immersed to the coating solution (5 mL 3-(Trimethoxysilyl)propylmethacrylate, 10 mL acetic acid, and 35 mL acetone) and incubated with shaking on an orbital shaker (SH30, FINEPCR) at 25 °C for 2 h. The glasses were again washed by acetone and distilled water and blew by nitrogen gas. The glasses were stored in a dry keeper (SANPLATEC co.) and used in 72 h. The cover glasses with solutions were sealed by nail polish and observed by LSM 800 laser scanning confocal microscope (Carl Zeiss, LSM800). Fluorescent images and DIC images were all processed by the Image J (National institutes of Health) software.

Partition coefficient (PC) analysis. Fluorescence mean intensities inside condensates were measured from condensates with >1 μm of radius *in vitro* and >0.1 μm^2 of size in cells. The brightest 10 condensates from a single cell were selected to calculate PCs in cells. For the fluorescence mean intensity outside condensates, a histogram of the signals of each pixel in the area except condensate parts was first obtained, and the highest 5% (1.96σ) signals are removed. Mean fluorescent signals of 95% remained pixels were measured as the outside mean intensity. PCs were obtained by dividing mean intensities inside of selected condensates with the outside mean intensities. 100 droplets from at least 3 independent experiments were selected for *in vitro* partition coefficient analysis. 30 or more cells were selected for partition coefficient analysis.

Fluorescence recovery after photobleaching (FRAP). FRAP were processed on glasses after sealing. Droplets were bleached with 20% of laser power for 1 sec interactive bleaching using a 561 nm laser line. Time-lapse images were collected 20 times in 12 second interval. Region of interest (ROI) was firstly set with the size of bleached region. At each time point, mean intensities of bleached region (I_R), neighboring unbleached region (I_U), and background region (I_B) with same ROI were measured. Recovered intensity ratio (I_t) were calculated by ($[I_R - I_B] / [I_U - I_B]$) at each time point, and fit to single exponential model ($I_t = I_\infty + (I_0 - I_\infty)e^{-kt}$) using Excel® (Microsoft® 2016), where I_0 is the ratio at the start of bleaching, I_∞ the ultimate recovery, and k the exponential constant. The mobile fraction of each curve was calculated by ($[I_B - I_0] / [I_\infty - I_0]$), where I_B is the initial ratio before bleaching. 15 droplets from at least 3 independent experiments were selected for the analysis of all diffusions.

Supporting Tables: Partition Coefficient Data Tables

Table S1 **Scaffold: NtFUS**

	PEG			BSA (+20 mg/ml PEG)			Dextran		
Clients	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
GFP-FUS	2.2 ± 0.41	4.6 ± 1.2	11.0 ± 1.5	2.3 ± 0.6	4.6 ± 0.9	8.5 ± 1.3	2.2 ± 0.3	3.3 ± 0.6	4.2 ± 0.7
GFP-F4CB	23.0 ± 7.0	49.1 ± 19.0	148.2 ± 61.1	22.9 ± 6.2	53.9 ± 16.7	81.1 ± 15.7	9.7 ± 4.4	12.5 ± 5.0	19.9 ± 7.5
GFP-F4CB-QR	14.6 ± 3.6	37.9 ± 10.1	159.8 ± 63.6	15.4 ± 2.6	25.1 ± 7.7	54.8 ± 7.6	-	-	-
GFP-F4CB-YR	3.0 ± 1.0	48.2 ± 38.8	77.5 ± 19.2	2.2 ± 0.5	3.8 ± 1.1	5.8 ± 2.0	-	-	-
GFP-LAF	6.5 ± 1.3	34.1 ± 11.5	216.6 ± 88.0	5.1 ± 1.8	8.3 ± 2.2	11.6 ± 2.3	3.2 ± 0.7	4.1 ± 1.2	6.6 ± 1.8
GFP-LAFK	1.5 ± 0.3	3.5 ± 1.0	31.2 ± 17.8	1.1 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.0 ± 0.06	1.1 ± 0.06	1.2 ± 0.2
GFP-LCD	2.0 ± 0.3	3.1 ± 0.4	9.5 ± 3.0	2.5 ± 0.2	3.5 ± 0.9	4.7 ± 1.0	1.5 ± 0.2	1.6 ± 0.3	2.2 ± 0.4
GFP-SC	1.4 ± 0.2	-	-	1.2 ± 0.2	-	-	-	-	-
GFP-FUS-SC	2.9 ± 0.7	-	-	3.1 ± 1.6	-	-	-	-	-
GFP-LAF-SC	8.2 ± 1.9	-	-	4.0 ± 1.1	-	-	-	-	-
GFP[-10]	0.9 ± 0.05	-	-	-	-	-	-	-	-
GFP[-2]	1.2 ± 0.07	-	-	-	-	-	-	-	-
GFP[+8]	4.8 ± 0.8	-	-	-	-	-	-	-	-
SUMO[-6]	1.5 ± 0.2	-	-	-	-	-	-	-	-
SUMO[+6]	2.1 ± 0.4	-	-	-	-	-	-	-	-

Table S2 **Scaffold: NtLAF**

	PEG			BSA (+20 mg/ml PEG)			Dextran		
Clients	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
GFP-FUS	2.1 ± 0.3	2.6 ± 0.6	2.9 ± 1.2	2.3 ± 0.8	2.5 ± 0.6	2.7 ± 0.9	-	2.9 ± 0.7	3.2 ± 0.6
GFP-F4CB	39.9 ± 7.9	58.0 ± 29.3	97.1 ± 45.4	58.7 ± 23.5	81.3 ± 17.4	85.2 ± 34.8	-	17.1 ± 5.2	20.8 ± 5.0
GFP-F4CB-QR	39.3 ± 13.0	50.8 ± 32.5	65.6 ± 26.3	74.8 ± 14.2	62.5 ± 24.4	79.5 ± 30.9	-	-	-
GFP-F4CB-YR	15.1 ± 3.0	23.8 ± 8.7	39.0 ± 14.8	5.2 ± 0.8	10.8 ± 2.1	10.3 ± 4.0	-	-	-
GFP-LAF	12.4 ± 4.1	31.3 ± 12.6	76.6 ± 30.1	6.6 ± 2.5	7.7 ± 1.7	6.3 ± 1.6	-	3.1 ± 1.0	5.4 ± 3.0
GFP-LAFK	1.7 ± 0.3	3.4 ± 0.7	15.2 ± 6.6	1.4 ± 0.2	1.4 ± 0.1	1.2 ± 0.1	-	1.1 ± 0.06	1.1 ± 0.07
GFP-LCD	2.5 ± 0.4	3.1 ± 0.9	4.3 ± 1.4	3.3 ± 0.7	4.2 ± 0.7	3.9 ± 1.2	-	2.7 ± 0.9	3.4 ± 1.1
GFP-SC	2.5 ± 0.5	-	-	1.8 ± 0.3	-	-	-	-	-
GFP-FUS-SC	3.7 ± 0.6	-	-	3.9 ± 0.9	-	-	-	-	-
GFP-LAF-SC	13.8 ± 2.7	-	-	8.4 ± 2.1	-	-	-	-	-
GFP[-10]	1.0 ± 0.04	-	-	-	-	-	-	-	-
GFP[-2]	1.2 ± 0.06	-	-	-	-	-	-	-	-
GFP[+8]	5.3 ± 0.8	-	-	-	-	-	-	-	-
SUMO[-6]	2.5 ± 0.2	-	-	-	-	-	-	-	-
SUMO[+6]	2.6 ± 0.3	-	-	-	-	-	-	-	-

Table S3 **Scaffold: PRM 5mer + SH3 5mer**

	PEG			BSA (+20 mg/ml PEG)			Dextran		
Clients	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
GFP-FUS	1.0 ± 0.05	1.7 ± 0.5	3.9 ± 0.8	1.1 ± 0.1	2.4 ± 0.9	2.1 ± 0.8	1.0 ± 0.08	1.0 ± 0.1	1.1 ± 0.1
GFP-LAF	4.3 ± 0.8	28.2 ± 11.1	161.6 ± 56.9	2.9 ± 0.5	4.4 ± 0.6	5.3 ± 1.6	1.8 ± 0.3	2.6 ± 0.8	3.4 ± 0.9
GFP-LAFK	1.4 ± 0.3	2.8 ± 0.9	98.3 ± 55.5	1.3 ± 0.2	1.7 ± 0.4	2.0 ± 0.6	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.1
GFP-LCD	1.3 ± 0.2	2.0 ± 0.4	5.3 ± 2.2	1.8 ± 0.3	3.2 ± 1.2	2.3 ± 1.0	1.1 ± 0.2	1.1 ± 0.2	1.4 ± 0.4
GFP[-10]	1.0 ± 0.1	-	-	-	-	-	-	-	-
GFP[-2]	1.5 ± 0.05	-	-	-	-	-	-	-	-
GFP[+8]	13.5 ± 3.4	-	-	-	-	-	-	-	-
SUMO[-6]	3.0 ± 0.6	-	-	-	-	-	-	-	-
SUMO[+6]	4.6 ± 1.0	-	-	-	-	-	-	-	-

Table S4 **Scaffold: NtFUS-D7N**

	PEG	BSA (+20 mg/ml PEG)	Dextran
Clients	50 mg/ml	50 mg/ml	50 mg/ml
GFP-FUS	2.4 ± 0.5	1.8 ± 0.4	-
GFP-F4CB	20.5 ± 7.0	10.3 ± 2.3	4.6 ± 1.6
GFP-LAF	6.0 ± 2.5	3.4 ± 0.7	2.1 ± 0.4
GFP-SC	9.7 ± 3.1	5.2 ± 2.0	4.1 ± 1.2
GFP-FUS-SC	17.2 ± 7.3	24.2 ± 12.0	-
GFP-LAF-SC	33.4 ± 10.9	25.1 ± 8.7	-

Table S5 Cell tests

	Scaffolds					
Clients	mCherry-Cry-FUS	mCherry-Cry-LAF	mCherry-Cry-FUS-D7N	mCherry-Cry-LAF-D7N	mCherry-Cry-FUS-D7Ns	mCherry-Cry-LAF-D7Ns
GFP	0.8 ± 0.6	1.3 ± 1.0	-	-	-	-
GFP-FUS	1.6 ± 0.5	1.8 ± 1.3	-	-	-	-
GFP-F4CB	6.0 ± 2.9	5.8 ± 1.8	4.5 ± 2.2	3.5 ± 1.6	-	-
GFP-F4CB-QR	4.0 ± 2.7	4.7 ± 3.4	-	-	-	-
GFP-F4CB-YR	2.2 ± 0.7	1.7 ± 0.7	-	-	-	-
GFP-LAF	1.9 ± 1.2	2.2 ± 1.2	1.5 ± 0.9	1.5 ± 0.7	-	-
GFP-LAFK	1.9 ± 1.3	2.4 ± 1.3	-	-	-	-
GFP-SC	-	-	7.4 ± 3.3	6.5 ± 4.4	3.9 ± 1.4	1.3 ± 0.9
GFP-2NB	1.7 ± 0.7	1.4 ± 0.9	-	-	-	-
GFP-2PB	6.1 ± 3.9	1.8 ± 1.1	-	-	-	-

Table S6 Figure 2b, 3a

	Crowding reagents	GFP-2NB	GFP-1NB	GFP-1PB	GFP-2PB	GFP-2PBK
NtFUS	PEG (50 mg/ml)	1.1 ± 0.06	1.0 ± 0.07	4.0 ± 1.1	5.8 ± 1.0	4.3 ± 1.8
NtLAF		15.2 ± 4.6	4.8 ± 0.9	21.3 ± 12.3	58.4 ± 11.0	4.8 ± 1.4

Table S7 Figure 5a

NtFUS (μM)	40	49	49.5	49.75
Binders (μM)	10	1	0.5	0.25
NtFUS-D7N + GFP-SC	8.4 ± 1.8	6.0 ± 1.3	4.5 ± 1.0	5.2 ± 1.0
NtFUS-D7Ns + GFP-SC	2.3 ± 0.4	1.4 ± 0.4	1.4 ± 0.2	1.3 ± 0.1

Table S8 Figure S5

	mini-CMV	CMV
mCherry-dronpa	3017.1 ± 2374.4	21008.2 ± 12085.2

Table S9 Figure S8

a

	PEG - 50 mg/ml	PEG - 100 mg/ml	PEG - 150 mg/ml
NtFUS	3.7 ± 1.2	5.6 ± 1.8	6.4 ± 0.8
NtLAF	6.1 ± 3.6	3.4 ± 1.3	2.5 ± 0.9

b

	BSA - 50 mg/ml (+20 mg/ml PEG)	BSA - 100 mg/ml (+20 mg/ml PEG)	BSA - 150 mg/ml (+20 mg/ml PEG)
NtFUS	1.2 ± 0.5	1.2 ± 0.6	1.0 ± 0.06
NtLAF	1.2 ± 0.1	1.5 ± 0.2	1.4 ± 0.2

Table S10 Figure S2a, S10

	Mobile Fraction
NtFUS	88.5 ± 6.6 %
NtLAF	79.8 ± 3.4 %
NtFUS-D7N	82.9 ± 14.4 %