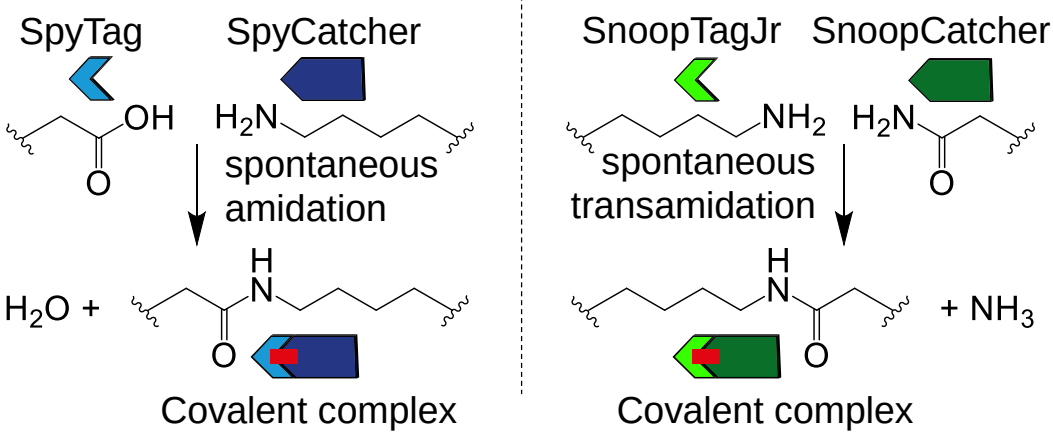
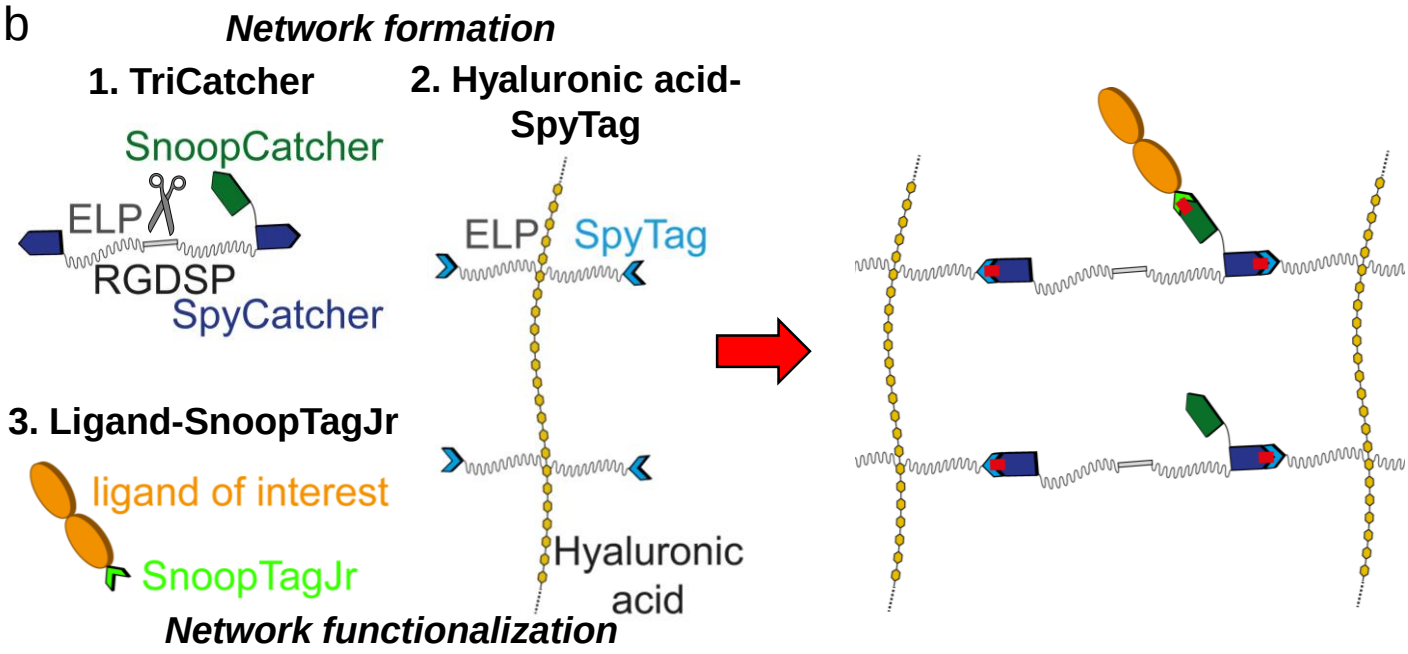
Figure 1

a



b



c

Linker protein	Central sequence
TriCatcher-ELP	LYAVTGRGRAPASSAPIATS
TriCatcher-RGDSP	LYAVTGRGDSPASSAPIATS
TriCatcher-MMP	LYARGRAPA**VPLS↓LYSG**IRATS
TriCatcher-RGDSP-MMP	LYARGDSPA**VPLS↓LYSG**IRATS

Figure 2

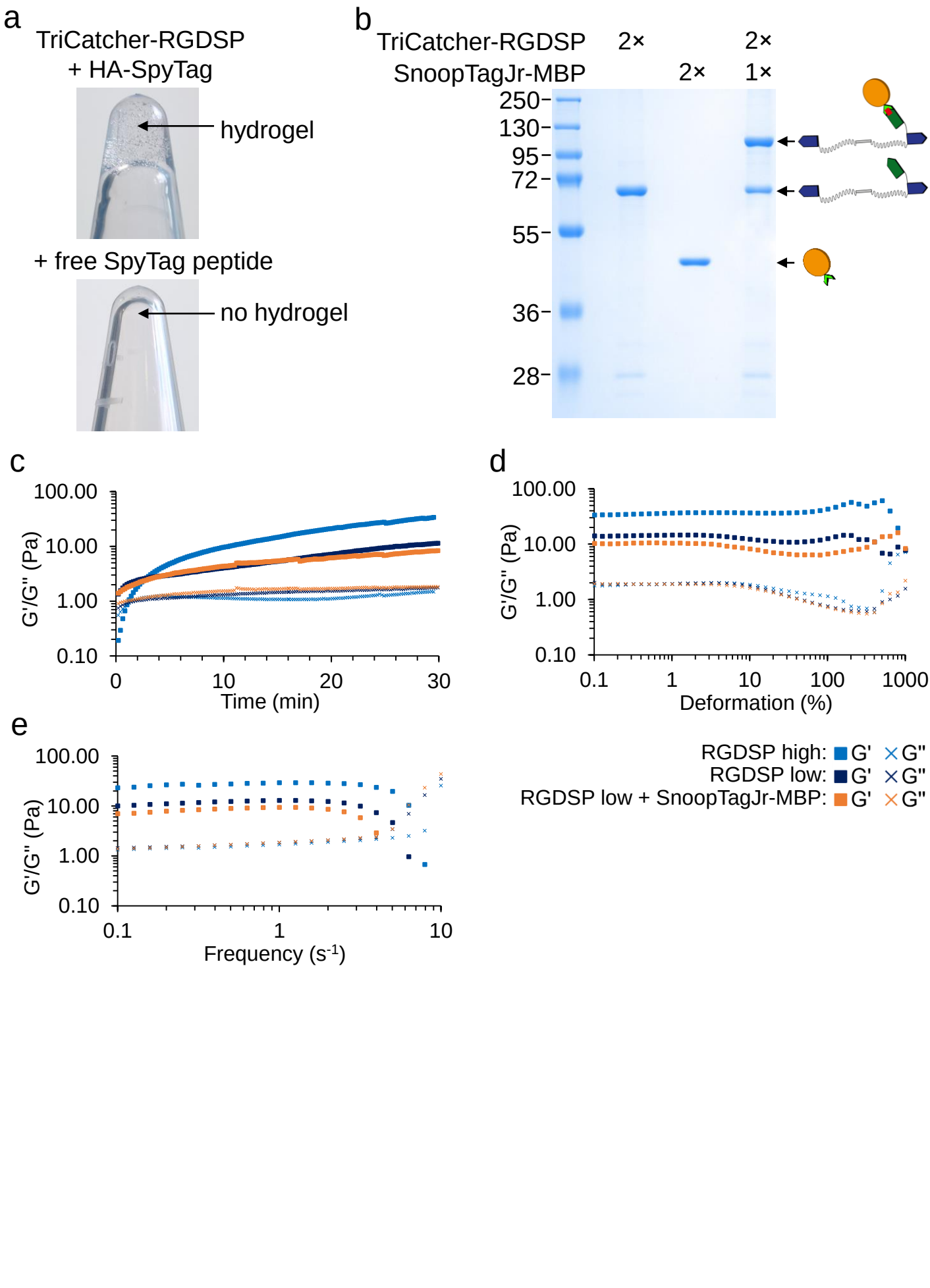
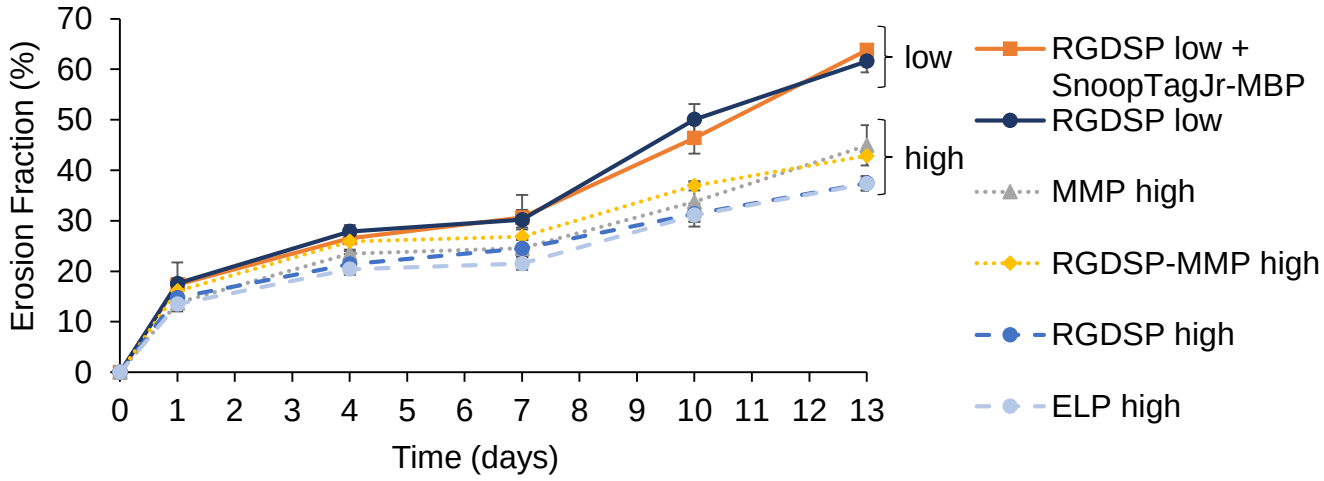
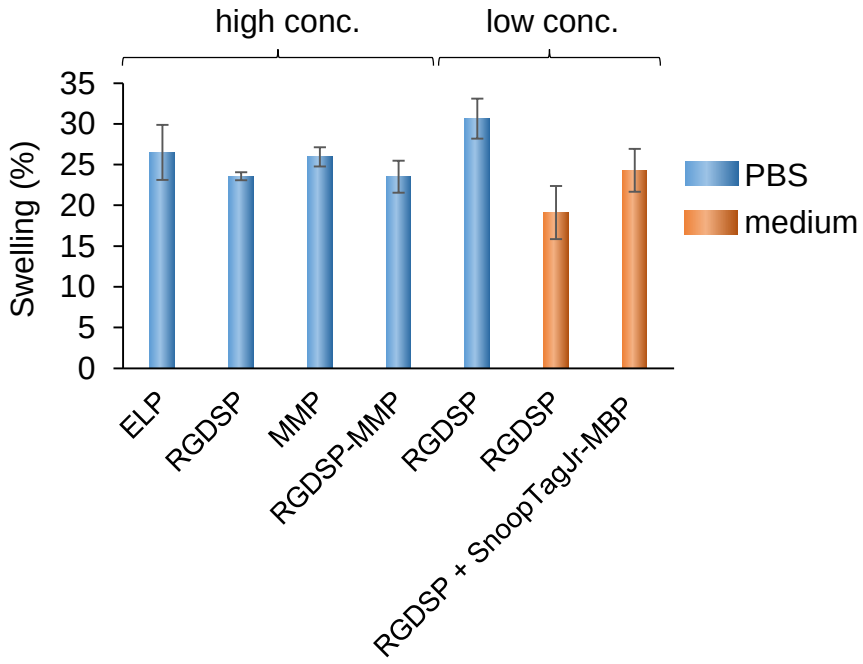

a
TriCatcher-RGDSP
+ HA-SpyTag
hydrogel
+ free SpyTag peptide
no hydrogel
b
TriCatcher-RGDSP
SnoopTagJr-MBP
2×
2×
2×
1×
250
130
95
72
55
36
28
c
100.00
10.00
1.00
0.10
G'/G" (Pa)
0
10
20
30
Time (min)
d
100.00
10.00
1.00
0.10
G'/G" (Pa)
0.1
1
10
100
1000
Deformation (%)
RGDSP high: G' G"
RGDSP low: G' G"
RGDSP low + SnoopTagJr-MBP: G' G"
e
100.00
10.00
1.00
0.10
G'/G" (Pa)
0.1
1
10
Frequency (s-1)

Figure 3

a


Erosion Fraction (%)
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Time (days)
low
high
RGDSP low + SnoopTagJr-MBP
RGDSP low
MMP high
RGDSP-MMP high
RGDSP high
ELP high

b


high conc.
low conc.
Swelling (%)
35
30
25
20
15
10
5
0
PBS
medium
ELP
RGDSP
MMP
RGDSP-MMP
RGDSP
RGDSP
RGDSP + SnoopTagJr-MBP

c

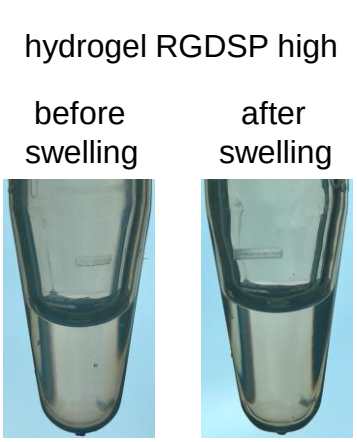

hydrogel RGDSP high
before swelling
after swelling

Figure 4

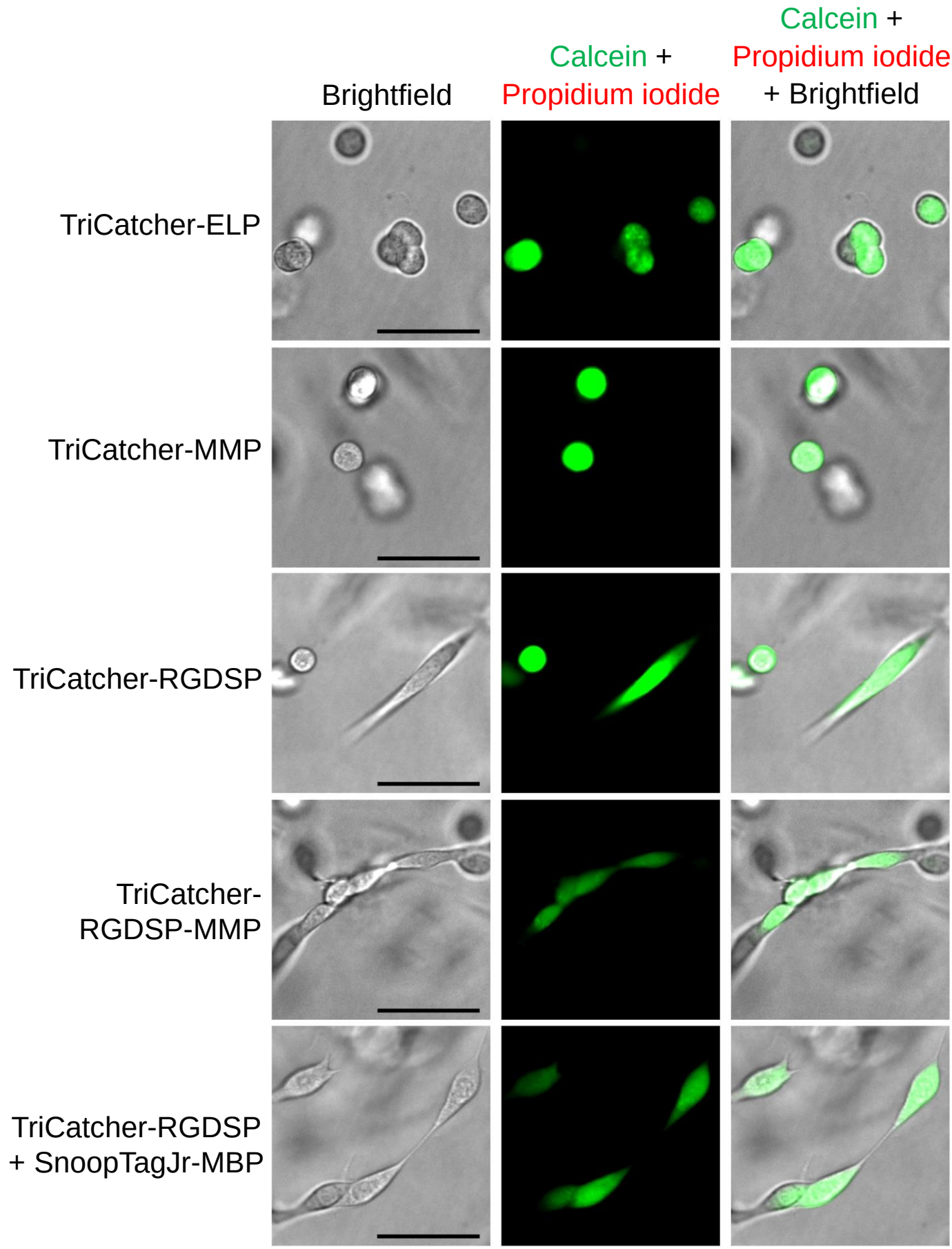
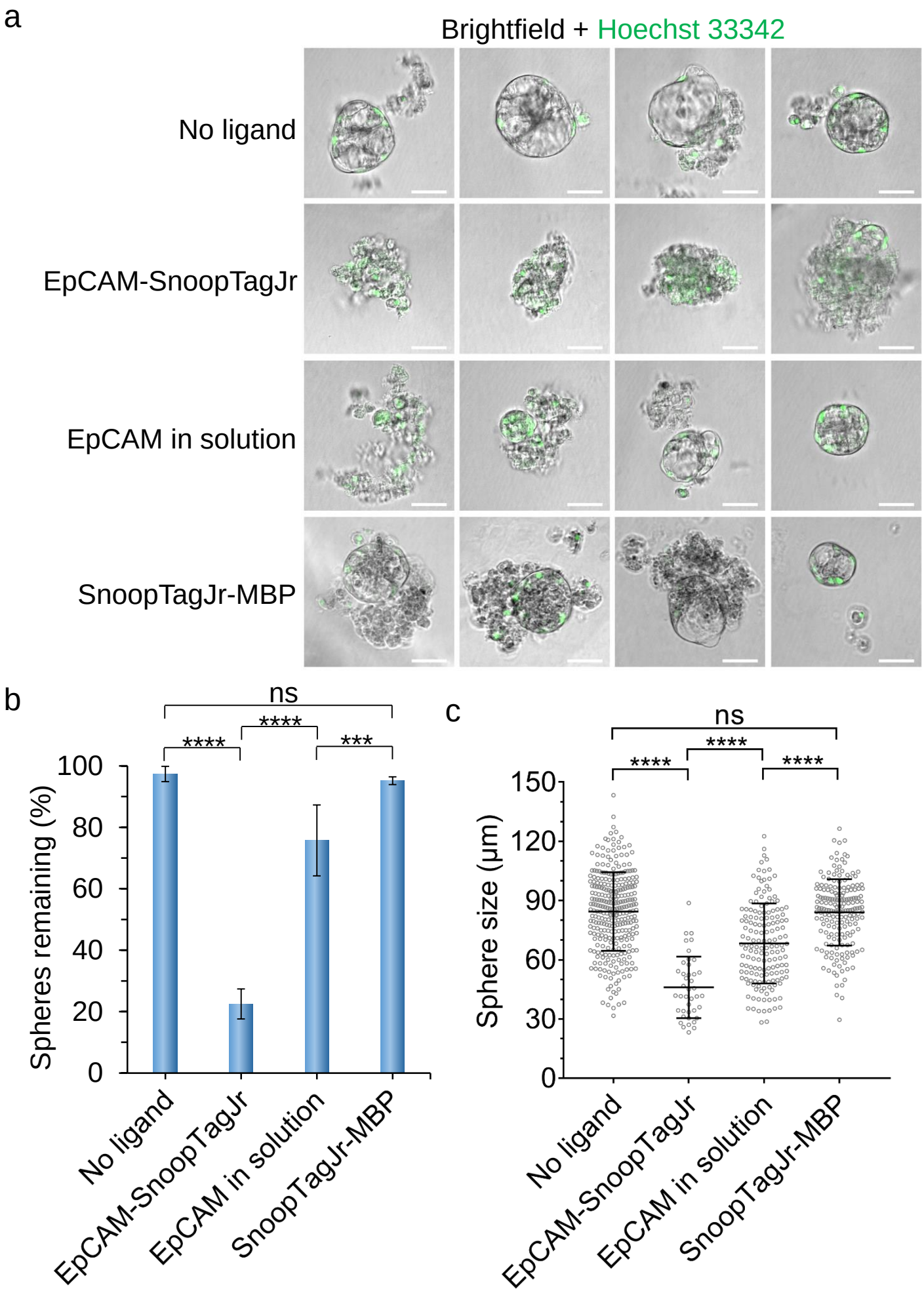

Brightfield
Calcein + Propidium iodide
Calcein + Propidium iodide + Brightfield
TriCatcher-ELP
TriCatcher-MMP
TriCatcher-RGDSP
TriCatcher-RGDSP-MMP
TriCatcher-RGDSP + SnoopTagJr-MBP

Figure 5


a
Brightfield + Hoechst 33342
No ligand
EpCAM-SnoopTagJr
EpCAM in solution
SnoopTagJr-MBP
b
ns
****
****
***
Spheres remaining (%)
100
80
60
40
20
0
No ligand
EpCAM-SnoopTagJr
EpCAM in solution
SnoopTagJr-MBP
c
ns
****
****
****
Sphere size (µm)
150
120
90
60
30
0
No ligand
EpCAM-SnoopTagJr
EpCAM in solution
SnoopTagJr-MBP

Supplements to:

Assembling and decorating hyaluronan hydrogels with twin protein superglues to mimic cell-cell interactions

Robert Wieduwild and Mark Howarth*

Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, United Kingdom

*Correspondence and requests for materials should be addressed to M.H. (email: mark.howarth@bioch.ox.ac.uk).

a) TriCatcher-ELP

MKGSSHHHHHHVDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDS**
SGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIDGSGGSGGQLD
GHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGEGVPGVG
VPGVGELYAVTGRGRAPASSAPIATSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGV
GVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHIKF**
SKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNG
KATKGDAHIGSPANLKALEAQKQKEQRQAAEELANAKKLKEQLEKGSHM**KPLRGAVFSLQKQHPDYPDIYGAIDQ**
NGTYQNVRTGEDGKLTFKNLSDGKYRLFENSEPAGYKPVQNKPIVAFQIVNGEVRDVTSIVPQDIPATYEFTNGK
HYITNEPIPPK*

b) TriCatcher-RGDSP

MKGSSHHHHHHVDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDS**
SGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIDGSGGSGGQLD
GHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGEGVPGVG
VPGVGELYAVTG**RGDSP**ASSAPIATSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGV
GVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHIKF**
SKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNG
KATKGDAHIGSPANLKALEAQKQKEQRQAAEELANAKKLKEQLEKGSHM**KPLRGAVFSLQKQHPDYPDIYGAIDQ**
NGTYQNVRTGEDGKLTFKNLSDGKYRLFENSEPAGYKPVQNKPIVAFQIVNGEVRDVTSIVPQDIPATYEFTNGK
HYITNEPIPPK*

c) TriCatcher-MMP

MKGSSHHHHHHVDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDS**
SGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIDGSGGSGGQLD
GHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGEGVPGVG
VPGVGELYARGRAPA**VPLSLYSG**IRATSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVP
GVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHI**
KFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTV
NGKATKGDAHIGSPANLKALEAQKQKEQRQAAEELANAKKLKEQLEKGSHM**KPLRGAVFSLQKQHPDYPDIYGAI**
DQNGTYQNVRTGEDGKLTFKNLSDGKYRLFENSEPAGYKPVQNKPIVAFQIVNGEVRDVTSIVPQDIPATYEFTN
GKHYITNEPIPPK*

d) TriCatcher-RGDSP-MMP

MKGSSHHHHHHVDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDS**
SGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIDGSGGSGGQLD
GHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGEGVPGVG
VPGVGELYA**RGDSP**A**VPLSLYSG**IRATSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVP
GVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDIPTTENLYFQGAM**VDTLSGLSSEQGQSGDMTIEEDSATHI**
KFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTV
NGKATKGDAHIGSPANLKALEAQKQKEQRQAAEELANAKKLKEQLEKGSHM**KPLRGAVFSLQKQHPDYPDIYGAI**
DQNGTYQNVRTGEDGKLTFKNLSDGKYRLFENSEPAGYKPVQNKPIVAFQIVNGEVRDVTSIVPQDIPATYEFTN
GKHYITNEPIPPK*

His-tag
TEV cleavage site
SpyCatcher with the reactive lysine underlined
RGDSP integrin-binding site
MMP cleavable linker
SnoopCatcher with the reactive asparagine underlined

Figure S1. Amino acid sequence of TriCatcher variants, with key at the bottom.

a) SpyTag-ELP-SpyTag

MKGSSHHHHHHVD**AHIVMVDAYKPTK**LDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPG
VGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELYAVTGRGRAPACSAPIATSVPGVGVPGVGVPGEGVPGVGVPG
VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLD**AHIVMVDAYKPTK**LEWK
K*

b) SpyTag-ELP-SpyTag DK

MKGSSHHHHHHVD**AHIVMVDAYKPTK**LDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPG
VGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELYAVTG*RGRAPA*CSAPIATSVPGVGVPGVGVPGEGVPGVGVPG
VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLLD**AHIVMVKAYKPTK**LEWK
K*

c) EpCAM-SnoopTagJr

MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRQEECVCENYKLAVNCFVNNNRQCQCTSVGAQNTVICSKLAAKCL
VMKAEMNGSKLGRRAKPEGALQNNDGLYDPDCDESGLFKAKQCNGTSMCWCVNTAGVRRTDKDTEITCSERVRTYW
IIIELKHKAREKPYDSKSLRTALQKEITTRYQLDPKFITSILYENNVITIDLVQNSSQKTQNDVDIADVAYYFEKD
VKGESLFHSKKMDLTVNGEQLDLDPGQTLIYYVDEKAPEFSMQGLKGSGGSGHHHHHHGSGGSG**KLGSIEFIKVNK**
GS*

d) EpCAM

MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRQEECVCENYKLAVNCFVNNNRQCQCTSVGAQNTVICSKLAAKCL
VMKAEMNGSKLGRRAKPEGALQNNDGLYDPDCDESGLFKAKQCNGTSMCWCVNTAGVRRTDKDTEITCSERVRTYW
IIIELKHKAREKPYDSKSLRTALQKEITTRYQLDPKFITSILYENNVITIDLVQNSSQKTQNDVDIADVAYYFEKD
VKGESLFHSKKMDLTVNGEQLDLDPGQTLIYYVDEKAPEFSMQGLKGSGGSGHHHHHHGS*

e) E-cadherin-SnoopTagJr

MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRDWVIPPISCPENEKGPFPKNLVQIKSNKDKEGKVFYSITGQGAD
TPPVGVFIIERETGWLKVTEPLDRERIATYTLFSHAVSSNGNAVEDPMEILITVTDQNDNKPEFTQEVFKGSVMEG
ALPGTSVMEVTATDADDDVNTYNAAIAYTILSQDPELPDKNMFTINRNTGVISVVTTGLDRESFPTYTLVVQAADL
QGEGLSTTATAVITVTDTNDNPPIFNPTTYKGQVPENEANVVITTLKVTDADAPNTPAWEAVYTILNDDGGQFVVT
TNPVNNDGILKTAKGLDFEAKQQYILHVAVTNVVPFEVSLTTSTATVTVDVLDVNEAPIFVPPEKRVEVSEDFGVG
QEITSYTAQEPDTFMEQKITYRIWRDTANWLEINPDTGAISTRAELDREDFEHVKNSTYTALIIATDNGSPVATGT
GTLLLILSDVNDNAPIPEPRTIFFCERNPKPQVINIIDADLPPNTSPFTAELTHGASANWTIQYNDPTQESIILKP
KMALEVGDYKINLKLMDNQNKDQVTTLEVSVCDCEGAAGVCRKAQPVEAGLQIPAGSGGSGGSGGSG**KLGSIEFIK**
VNKGSGHHHHHHGS*

His-tag
TEV cleavage site
SpyTag with the reactive aspartic acid or DK mutation underlined
Reactive cysteine
Leader sequence
Extracellular portion of EpCAM
Extracellular portion of E-cadherin
SnoopTagJr with the reactive lysine underlined

Figure S2. Amino acid sequence of SpyTag fusions and adhesion proteins, with key at the bottom.

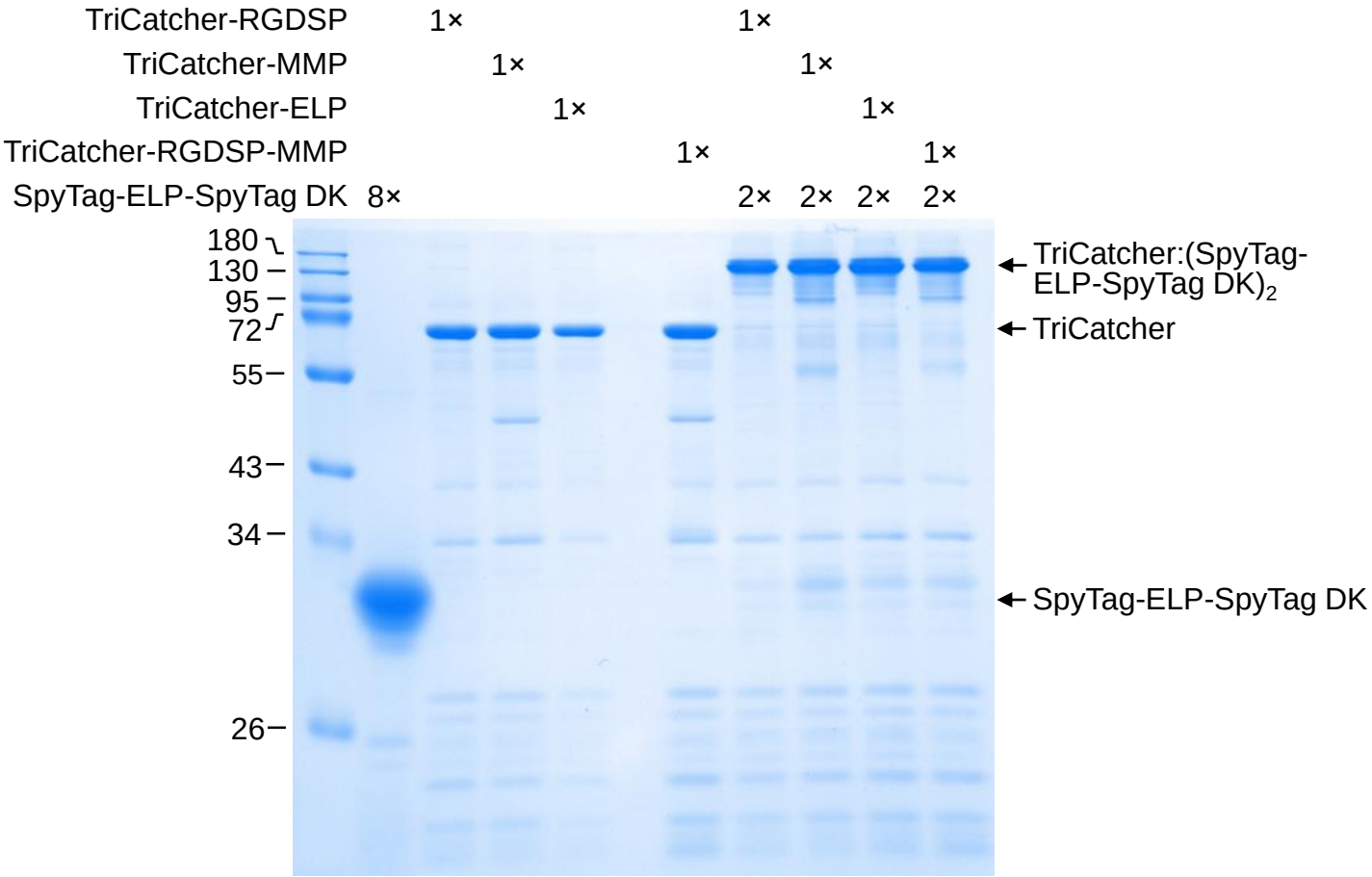


Figure S3. Efficient covalent reaction of 80 µM TriCatcher variants with 160 µM SpyTag-ELP-SpyTag DK at a molar ratio of 1:2. 1× refers to 2 µM . Reactions were incubated for 12 h in PBS at 4 °C, boiled in SDS and reducing agent, and analyzed by SDS-PAGE with Coomassie staining.

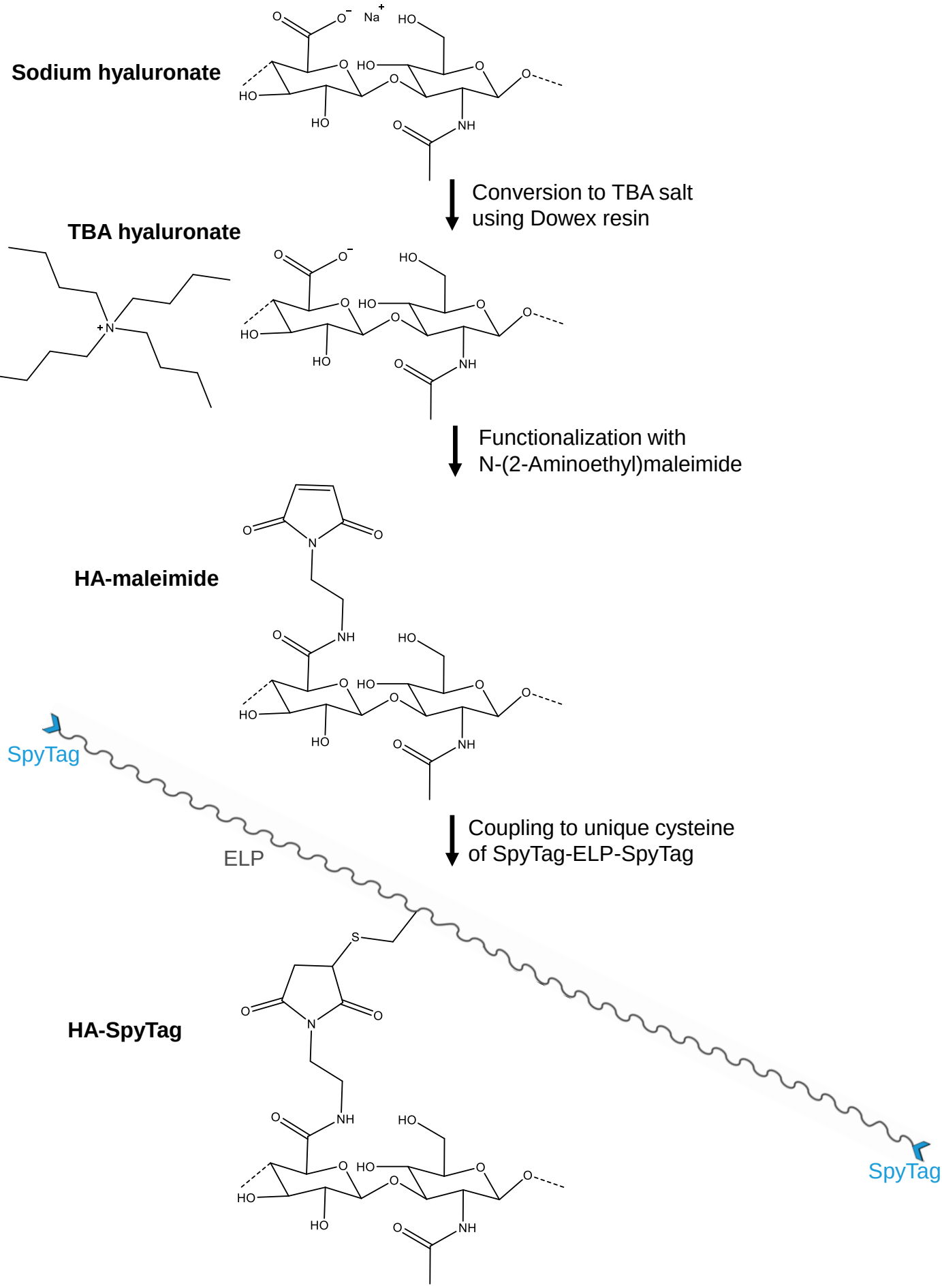


Figure S4. Route for coupling of hyaluronic acid to SpyTag-ELP-SpyTag.

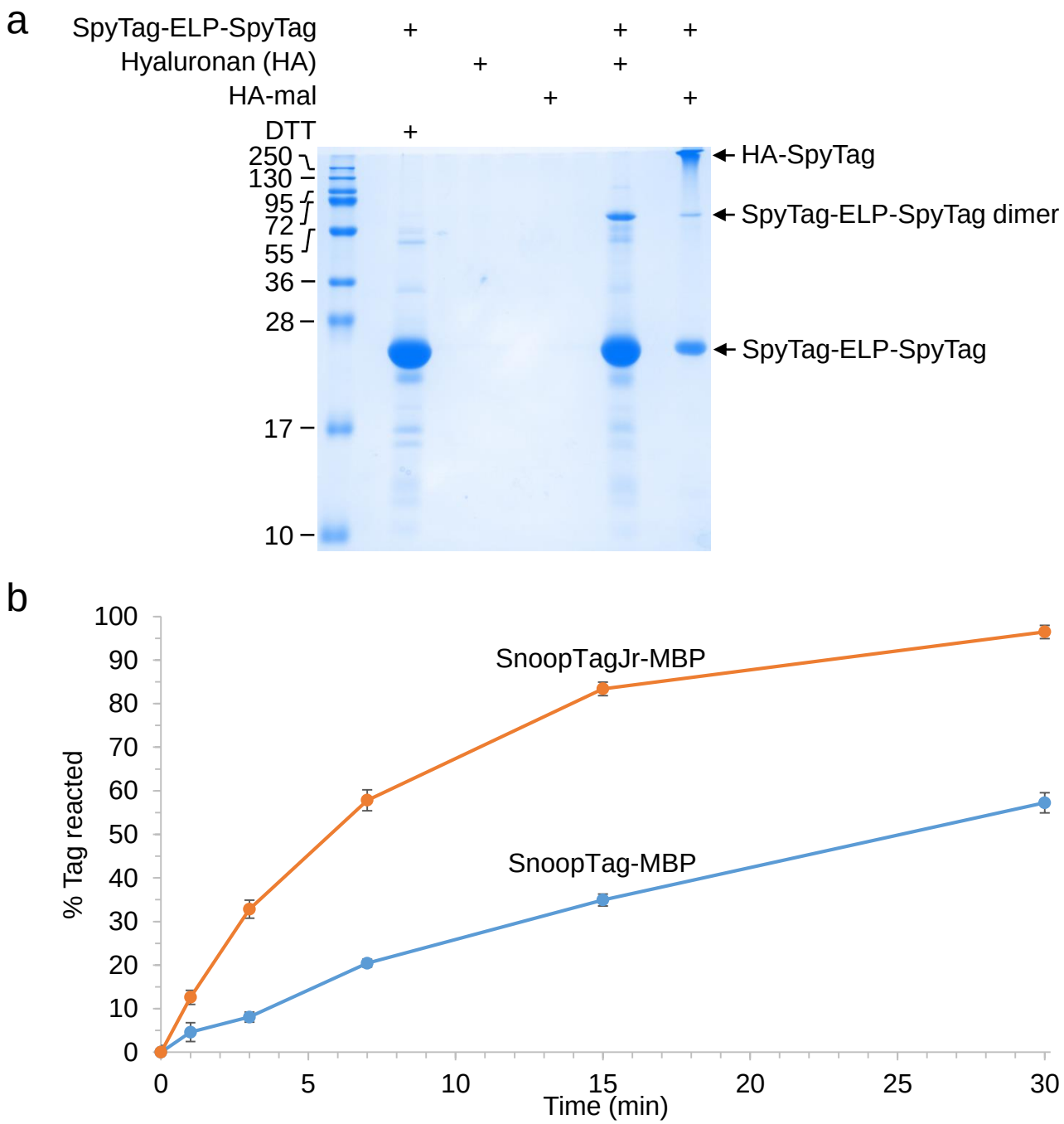


Figure S5. Analysis of coupling of SpyTag-ELP-SpyTag to HA-mal and reaction of SnoopTag-MBP/SnoopTagJr to SnoopCatcher. **a)** SpyTag-ELP-SpyTag and HA-mal were mixed to ~4 mg/mL of each component for 2 h, before analysis by SDS-PAGE with Coomassie staining. Uncoupled HA is used as a negative control. Neither HA nor HA-mal are stained by Coomassie. **b)** SnoopTagJr reacted faster than SnoopTag with SnoopCatcher. SnoopCatcher (10 µM) was incubated for the indicated time with SnoopTagJr-MBP or SnoopTag-MBP (each 5 µM) in PBS at pH 7.4 and the percentage of Tag reacted was analyzed by SDS-PAGE with Coomassie staining (mean of triplicate ± 1 s.d.).

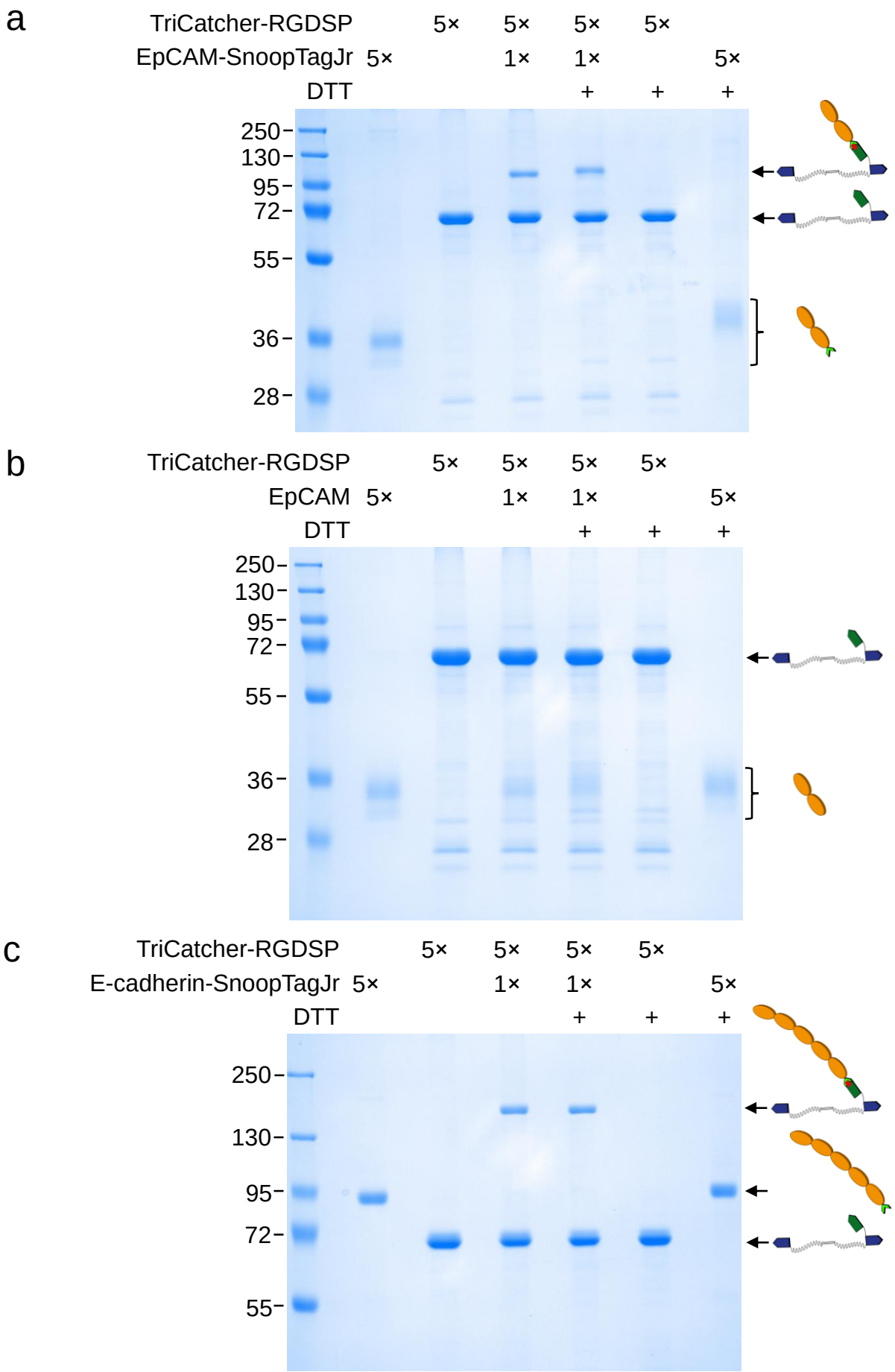


Figure S6. TriCatcher reaction with adhesion proteins. **a**) Reaction of TriCatcher-RGDSP with EpCAM-SnoopTagJr at a molar ratio of 5:1 (1× = 0.4 µM), incubated for 12 h in PBS at 4 °C, prior to boiling and SDS-PAGE with Coomassie staining. **b**) No reaction of TriCatcher with EpCAM lacking tag. Analyzed as in (a). **c**) Reaction of TriCatcher-RGDSP with E-cadherin-SnoopTagJr, analyzed as in (a).

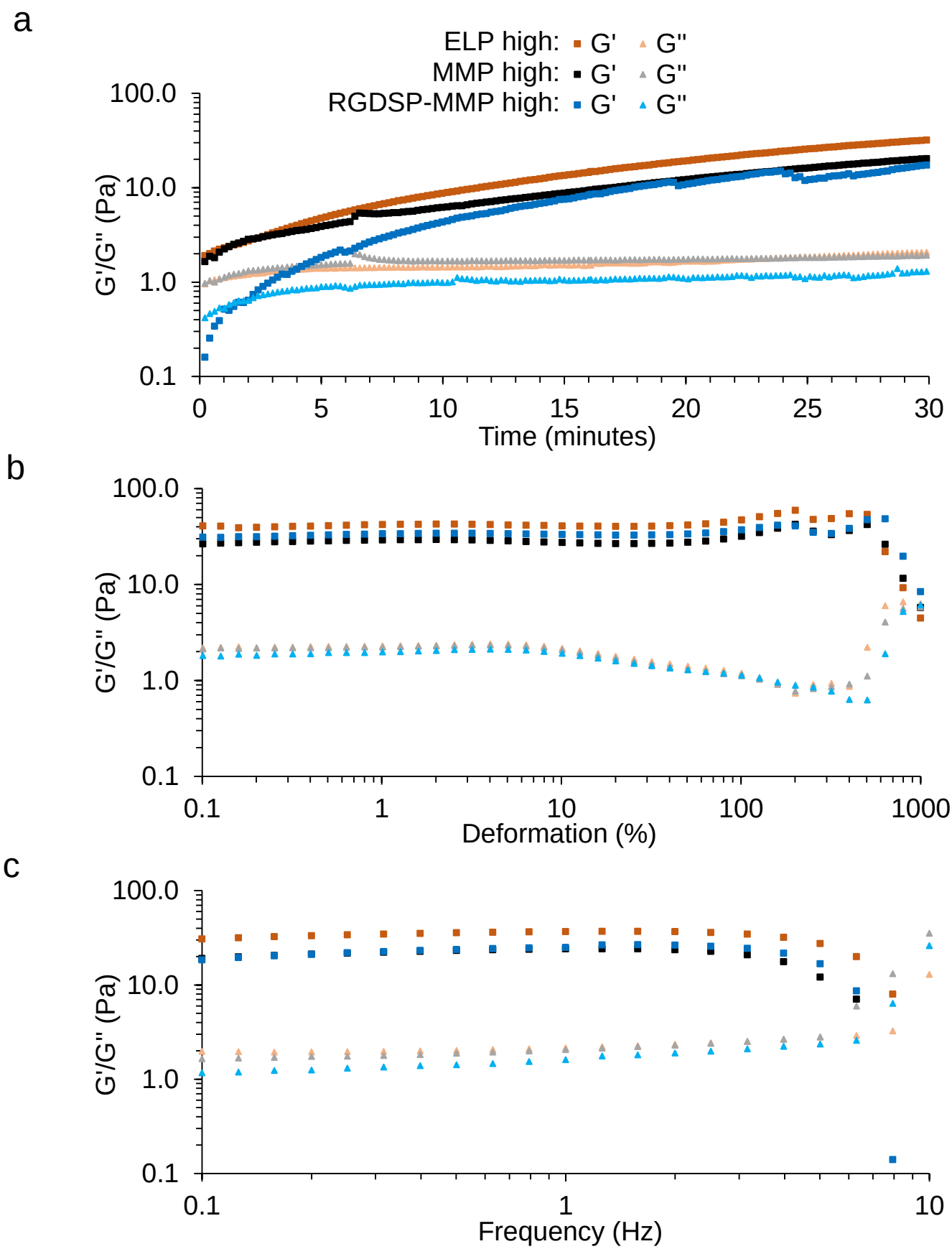


Figure S7. Rheological analysis of hydrogels with different TriCatchers. Hydrogels were formed from TriCatcher and HA-SpyTag at high concentration (each 80 µM) or low concentration (each 53 µM) in PBS or growth medium. **a**) Time-dependence of storage modulus (G') and loss modulus (G''). **b**) Strain sweep of hydrogels at 1 Hz frequency. **c**) Frequency sweep using 1 % deformation.

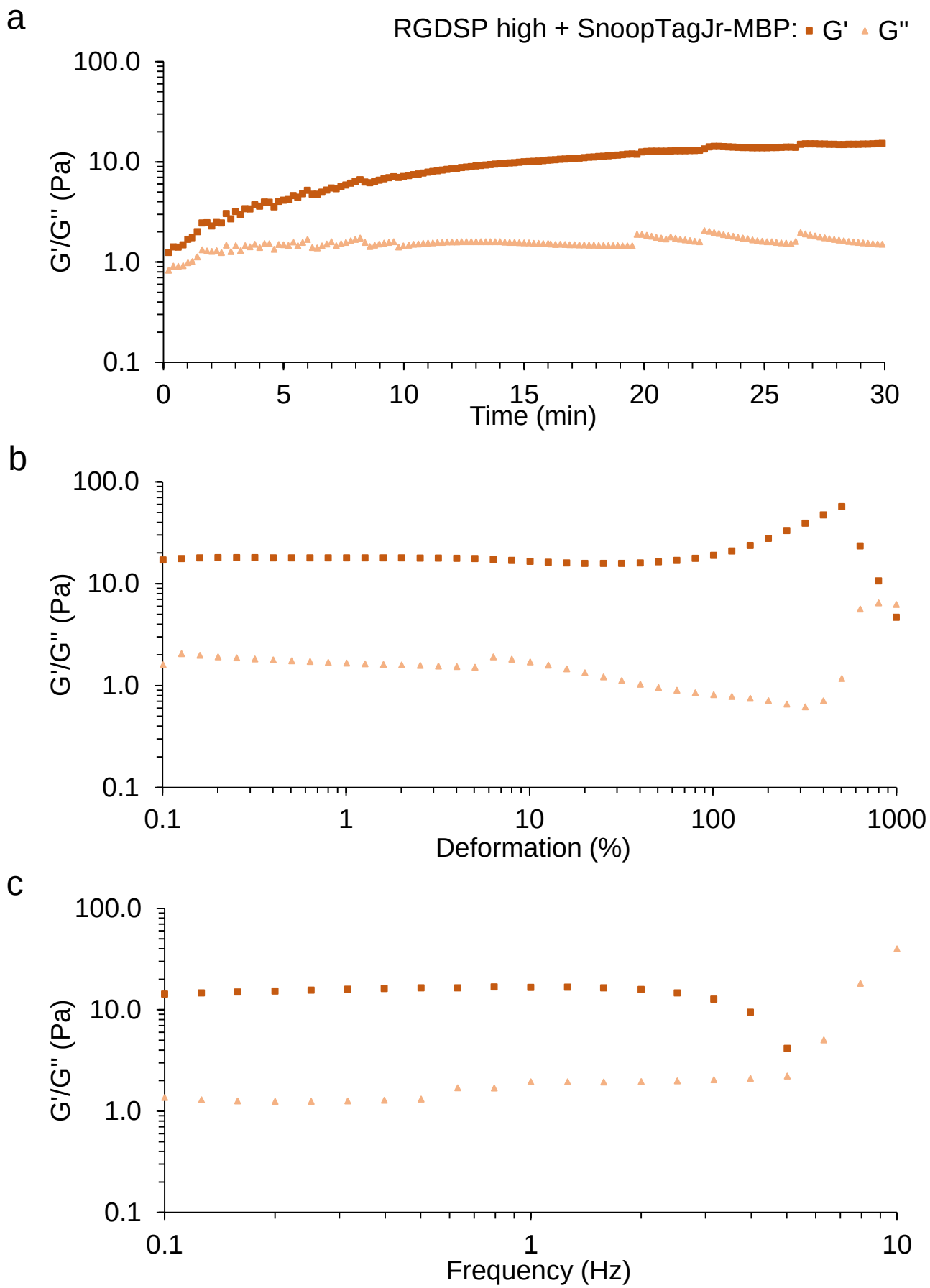


Figure S8. Rheological analysis of hydrogels with coupling of SnoopTagJr-MBP prior to hydrogel formation. Hydrogels were formed from TriCatcher and HA-SpyTag at high concentration (each 80 µM) in growth medium. **a**) Time-dependence of storage modulus (G') and loss modulus (G''). **b**) Strain sweep of hydrogels at 1 Hz frequency. **c**) Frequency sweep using 1 % deformation.

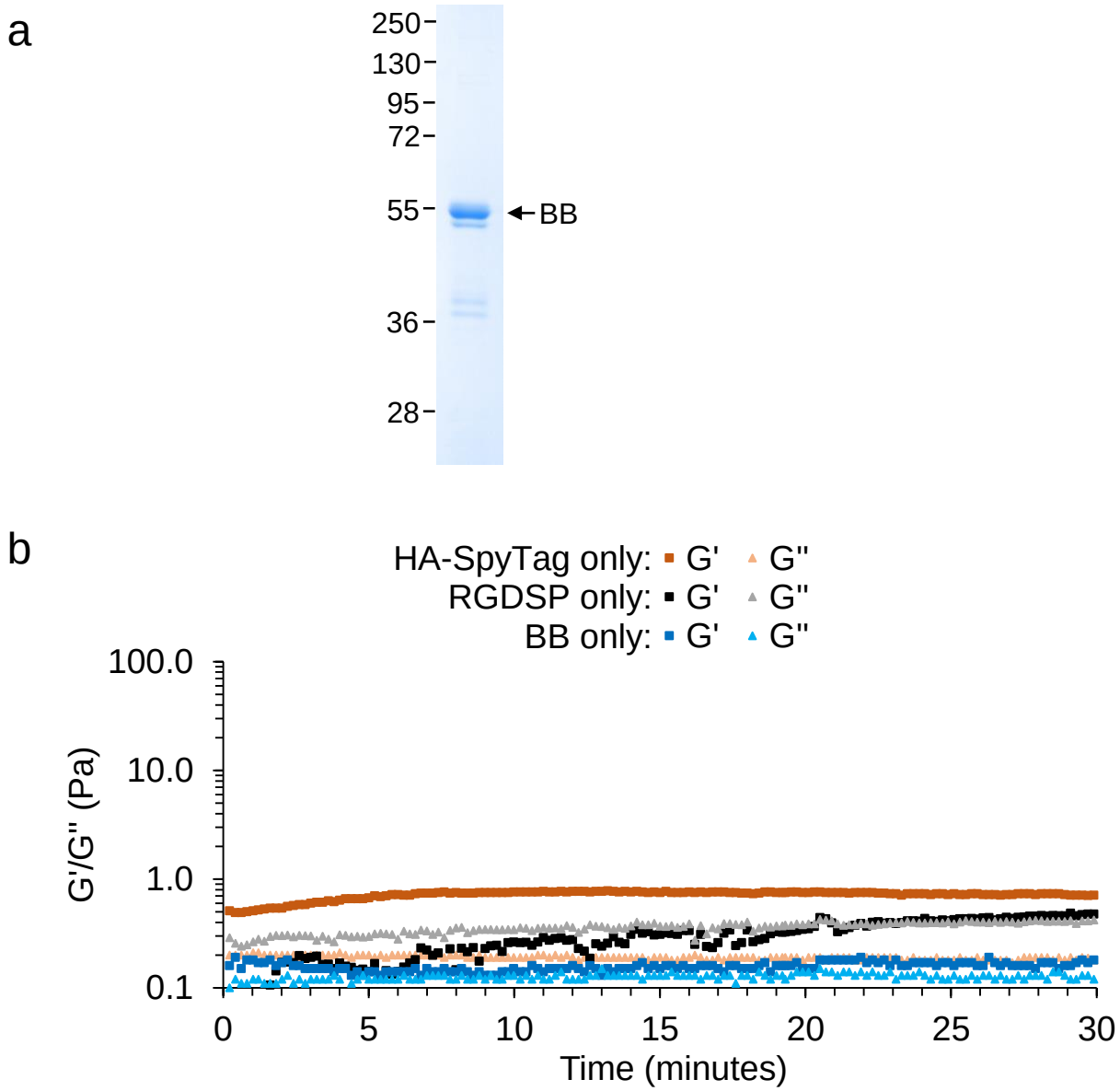


Figure S9. Purified BB and rheological analysis of individual hydrogel components. **a)** SDS-PAGE with Coomassie staining of purified BB at 6 µM. **b)** Time-dependence of storage modulus (G') and loss modulus (G''), using 80 µM TriCatcher-RGDSP, 120 µM BB or 120 µM HA-SpyTag individually.

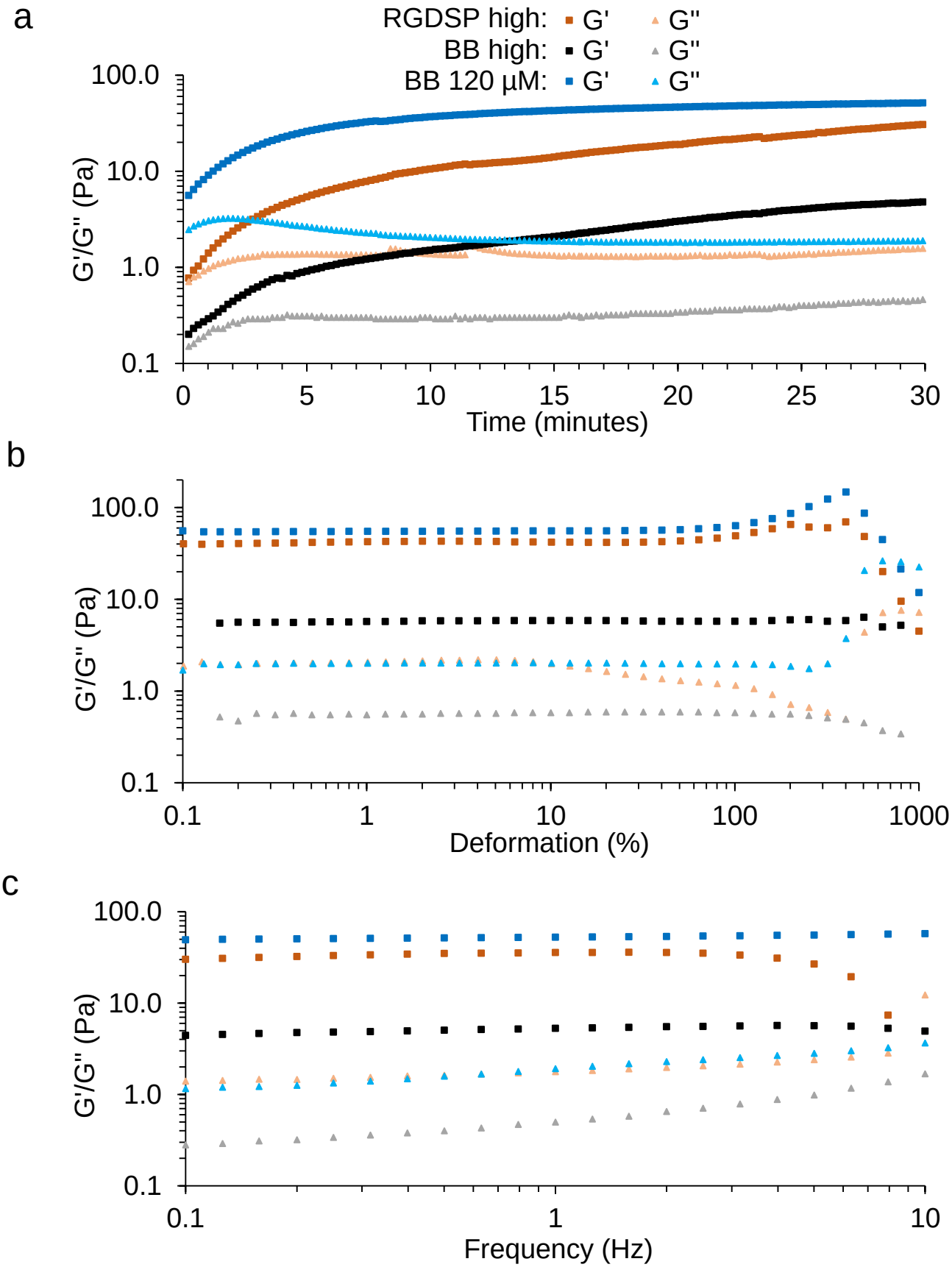


Figure S10. Rheological analysis of hydrogels with BB (SpyCatcher-ELP-SpyCatcher) compared to TriCatcher-RGDSP. Hydrogels were formed in PBS from 80 µM HA-SpyTag with 80 µM TriCatcher-RGDSP (high), or 80 µM HA-SpyTag with 80 µM BB (high). Additionally, hydrogels were formed in PBS from 120 µM HA-SpyTag with 120 µM BB. **a**) Time-dependence of storage modulus (G') and loss modulus (G''). **b**) Strain sweep of hydrogels at 1 Hz frequency. **c**) Frequency sweep using 1 % deformation.

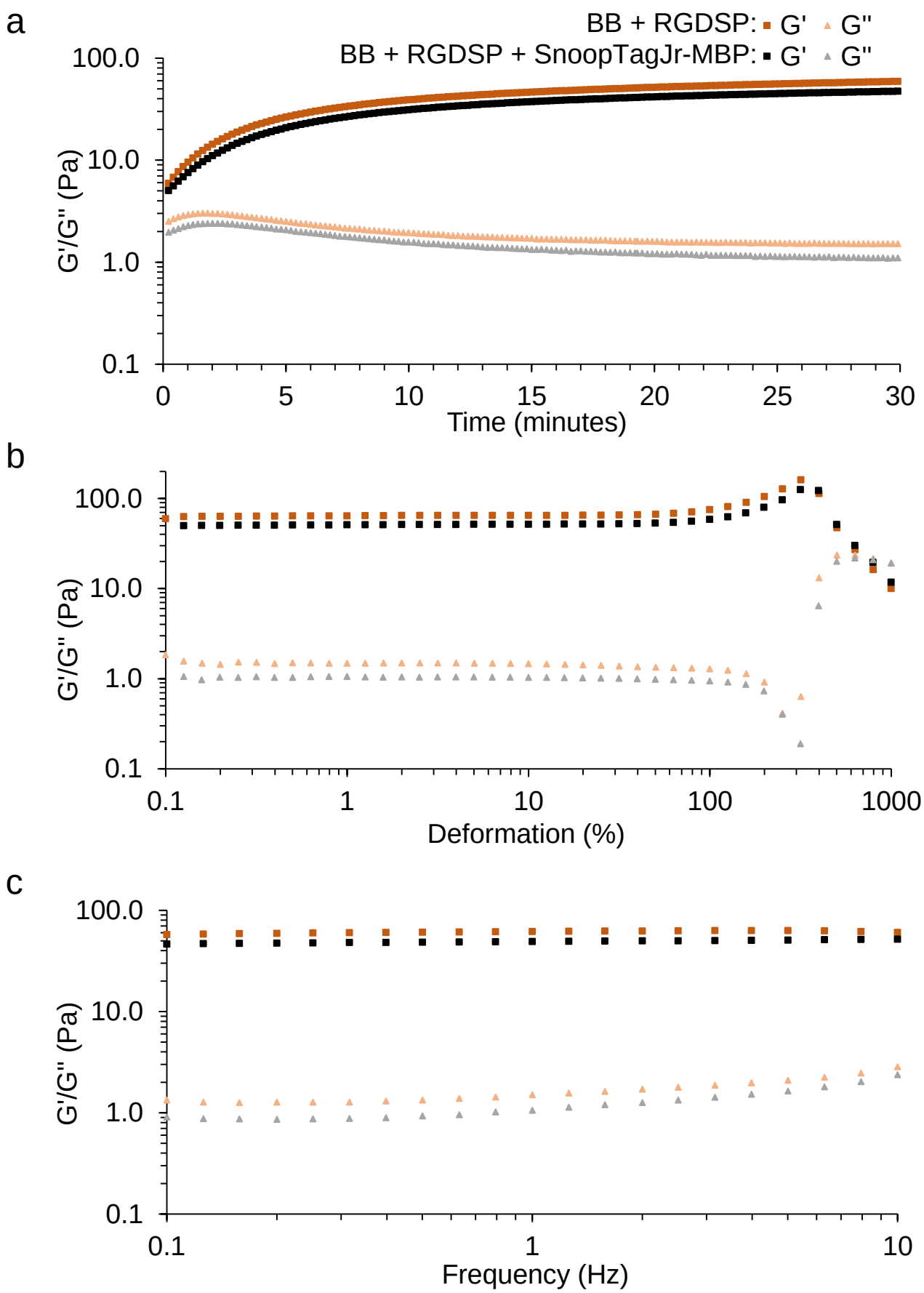


Figure S11. Rheological analysis of hydrogels cross-linked with BB (SpyCatcher-ELP-SpyCatcher) and TriCatcher-RGDSP combined. Hydrogels were formed in PBS from 72 µM BB, 48 µM TriCatcher-RGDSP and 120 µM HA-SpyTag, with or without 24 µM SnoopTagJr-MBP. **a**) Time-dependence of storage modulus (G') and loss modulus (G''). **b**) Strain sweep of hydrogels at 1 Hz frequency. **c**) Frequency sweep using 1 % deformation.

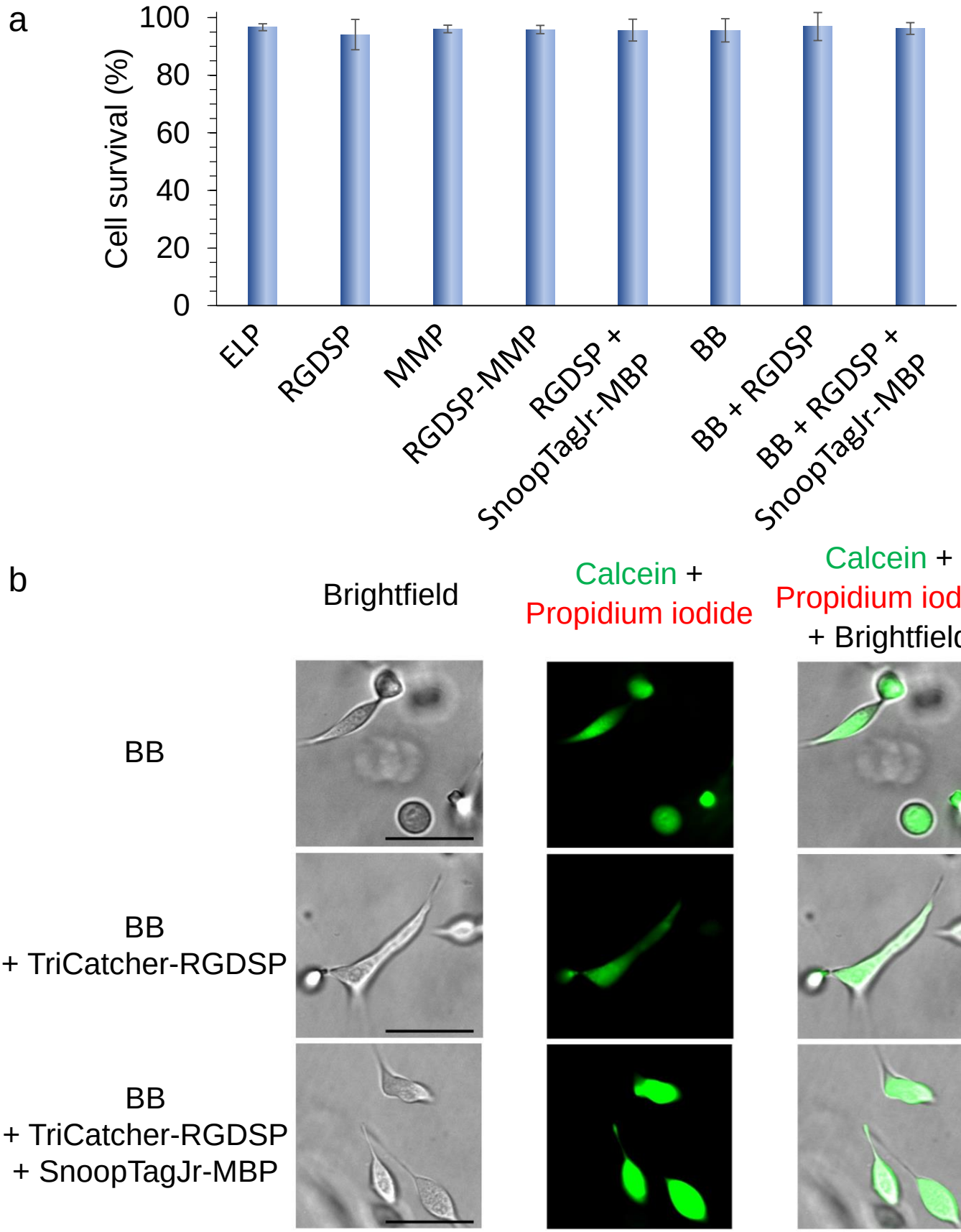


Figure S12. Cell maintained high viability in the hydrogel. Mouse 3T3 fibroblasts were encapsulated in hydrogels containing varying hydrogel components, cultured for 1 d, and analyzed by confocal microscopy. **a)** Cell survival was determined using calcein AM ester viability and propidium iodide dead staining from a 100 µm z-stack (mean ± 1 s.d. from 12 z-stacks) **b)** The brightfield image, calcein AM ester viability staining (green) and propidium iodide dead staining (red) of a single z-section are shown. Scale bar 50 µm.

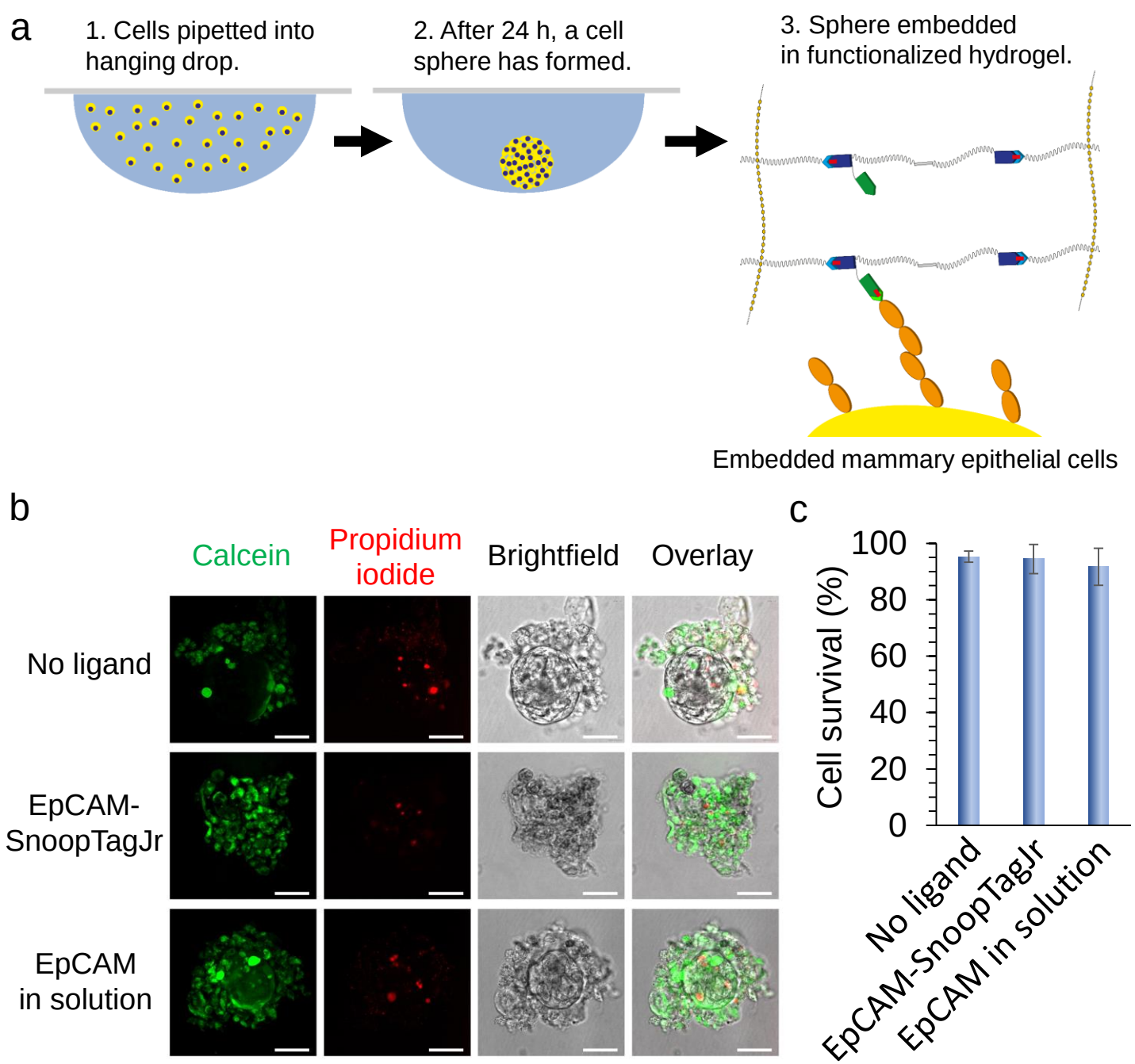


Figure S13. Live/dead staining of cell spheres after hydrogel culture. **a)** Cartoon of cell sphere generation and embedding. **b)** Spheres were grown for 6 d in the hydrogel functionalized with the indicated EpCAM constructs or no ligand. MCF 10A cells were stained with the viability stain calcein AM ester (green, left column) and dead cells were detected with propidium iodide (red, middle-left column), using a confocal microscope showing the z-projection of a 100 µm stack. Brightfield images (grayscale, middle-right column) were acquired and then all images were overlaid (right column). Scale bar 50 µm. **c)** Cell survival of MCF 10A cells in hydrogels with the indicated ligand was determined using calcein AM ester viability and propidium iodide dead staining from a 100 µm z-stack. Mean of n=15 ± 1 s.d. (no ligand); mean of n=11 ± 1 s.d. (EpCAM-SnoopTagJr, EpCAM in solution).

Movie S1: Formation of robust hydrogel in seconds. Hydrogel was formed with 80 µM each of HA-SpyTag and TriCatcher-RGDSP.