Supporting Information

Investigating Fluorescence Dyes in Fluorescence-Assisted Screenings

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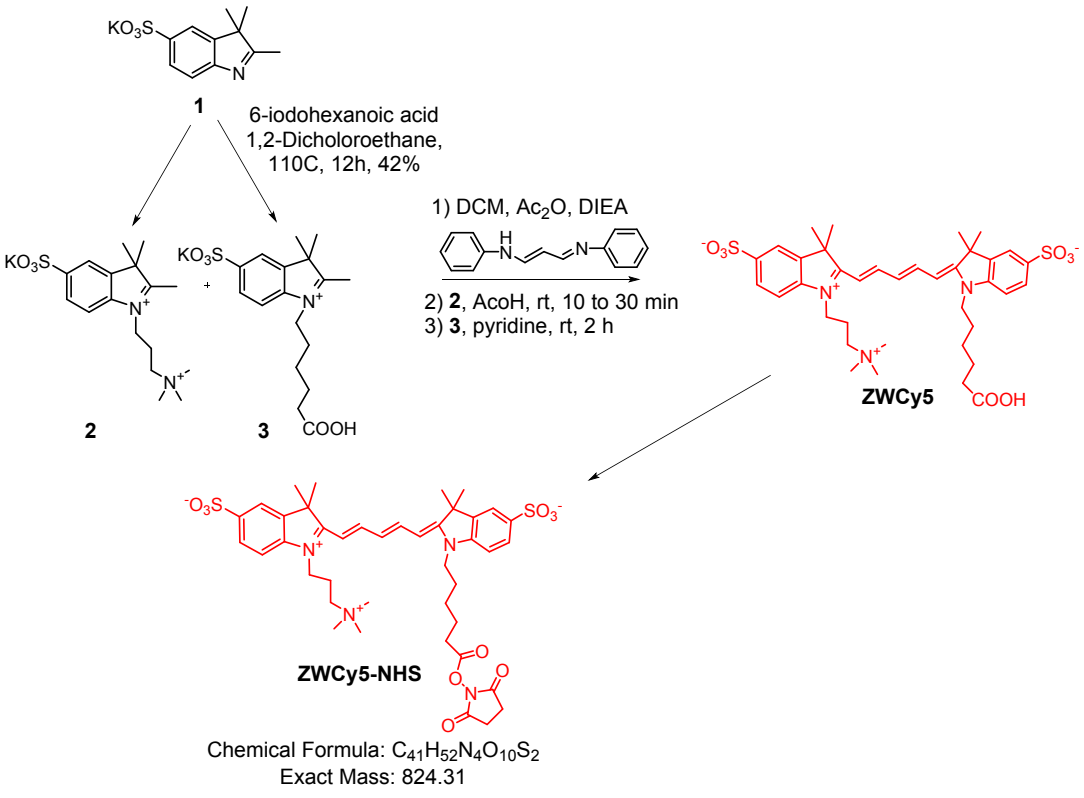
Experimental section

Materials. *h*CAII was purchased from Sigma-Aldrich. N-methylpyrrolidone (NMP), diethylether, dichloromethane (DCM) and Fmoc-γ-Abu-OH (GABA) were purchased from Merck. Fmoc-protected D-amino acids (Fmoc-AAs) were purchased from GL Biochem Ltd (Shanghai, China). TentaGel S amino resin was purchased from Rapp Polymere. α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from Bruker. EZ-Link NHS-Biotin reagent was purchased from Thermo Scientific. *h*CAII (Aldrich), *b*CAII (Aldrich), PSA (Fitzgerald), AFP (YoProtein), CRP (Merck), his-tagged Ki67 (MyBiosource) were purchased from commercial sources. Unless otherwise specified, chemicals were purchased from Aldrich. MALDI-MS and -MS/MS spectra were obtained using ultrafleXtreme™ TOF/TOF (Bruker). Microwave-assisted CNBr-based cleavage reactions were performed by a household microwave oven (model R-248J, 800 W, 2450 MHz) from Sharp Inc. The PEAKS software was purchased from Bioinformatics Solutions Inc. The purification of bulk peptides was done by a preparative HPLC system from Gilson on a C_{18} reversed phase preparative column (Kromasil® from AkzoNobel, 5 µm, 250 × 30 mm).

Synthesis of ZWCy5



1) Synthesis of **2**[1]

A mixture of **1** (7.17 g, 35.4 mmol) and (3-bromopropyl)trimethylammonium bromide (10.5 g, 40.0 mmol, 1.2 eq) in toluene (60 mL) was heated at 130°C for 72 h under a nitrogen atmosphere. The mixture was cooled to room temperature and the solvent was decanted. The crude product was crystallized from methanol and methyl *t*-butyl ether to afford a pink powder, which was used in the next step without any purification.

2) Synthesis of **3**[2]

A mixture of **1** (1.1 g, 3.97 mmol) and 6-iodohexanoic acid (0.98 g, 4.05 mmol, 1.1 eq) in 1,2-dichlorobenzene (10 mL) was heated at 110°C overnight under a nitrogen atmosphere. The mixture was cooled and solvent was decanted. Solid was triturated with 2-propanol until a free pink powder (0.63 g, 42%) was obtained, which was used in the next step without any purification.

3) Synthesis of **ZWCy5**

Malonaldehyde dianilide hydrochloride (17 mg, 66 µmol) is suspended in dry dichloromethane (100 µL). Then acetic anhydride (13 µL, 2 eq) and DIEA (11 µL, 1 eq) were added sequentially and the mixture is stirred at room temperature for 3 hours. Then a solution of 2 (25 mg, 66 µmol, 1eq) in AcOH (100 µL) was added to the previous solution and stirred at room temperature for 10 to 30 min1. The color turns from the yellow to the green. Then a solution of 3 (26 mg, 91 µmol) in pyridine (200 µL) is added and mixture is stirred for 2 h at room temperature. The color turns from the green to the dark blue. Solvents are removed and the crude is purified by RP-HPLC to get a blue amorphous powder (15 mg, 32%).

4) Synthesis of **ZWCy5-NHS**

ZWCy5 (100 mg, 0.13 mmol) is suspended in dry DMF (30mL) under nitrogen atmosphere. Then NHS (120 mg, 1.04 mmol, 8 equiv.) and EDC (260 mg, 1.30 mmol, 10 equiv) were added sequentially. The mixture is stirred for 6 hours at room temperature under inert atmosphere. Then ethyl acetate is added until the product precipitate. Product is filtrated, redissolved in acetonitrile and purified by RP-HPLC to get **ZWCy5-NHS** ester as blue amorphous powder (79 mg, yield 70%).

Analytical RP-HPLC: tR= 3.971min compared to tR=3.940 min for **ZWCy5**

HR-MS: *m/z* = 847.3040, calculated for $C_{41}H_{52}N_4NaO_{10}S_2$ [M + Na] = 847.3017.

Construction of peptide libraries. Random OBOC peptide libraries were synthesized using our reported method.[3,4] For the solutions of Fmoc-isoleucine and Fmoc-glutamine, both contained 10 mol% of glycine each for discrimination of isobaric residues in the semi-automatic peptide sequencing.

Labeling of protein with dyes. To label *h*CAII with various dyes such as **ZW**, **AL** (Alexa Fluor® 647) and **DY** (DyLight 650), 500µl of a *h*CAII solution (2 mg/mL) in PBS (pH 8.3) was mixed with 5 molar equivalents of NHS-activated dye dissolved in DMSO (10 mg/ml). The mixture was incubated for 1 h at room temperature under dark conditions. The dye-labeled *h*CAII was purified by using size exclusion chromatography. Upon purification the resulting dye-labeled *h*CAII was characterized by UV-vis spectroscopy and SDS-PAGE. Protein concentration was determined by UV absorbance at 280 nm.

Library screening. For the screen, 100 mg of library resin was transferred into an Alltech vessel (8ml, equipped with a filer) and pre-incubated in a blocking solution, comprising of 0.05% NaN_3, 0.1% Tween 20, and 0.1% BSA in PBS buffer (pH 7.4), for 1 h on a 360° shaker at 25 °C. Dye-labeled *h*CAII was added to a final concentration of 100 nM and the library was incubated for 4 h on a 360° shaker at 25 °C. The liquid was drained and the resulting beads were washed three times with the blocking solution and three times with 0.1% Tween 20 in PBS buffer sequentially. After washing, the beads were transferred into a sample vessel of COPAS Plus (Union Biometrica) and diluted with 200 mL of PBS buffer with 0.1% Tween 20 (pH 7.4). Two-step sorting was carried out to sort out positive beads. In the second sort, positive beads were directly sorted into a 96 titer well plate with cone-shaped wells.

CNBr-based cleavage of peptides from single beads. The 96 well plate with sorted beads was purged by argon for 15 min and then CNBr (10 µL, 0.50 M in 0.2 N HCl solution) was added into each well. After additional purging by argon/nitrogen for 15 min, the 96 well plate was sealed and placed under microwave radiation for 1 min. The resulting solution was concentrated under centrifugal vacuum for 2.5 hr at 45 °C.[5]

MALDI-MS and MS/MS analysis of peptides cleaved from single beads. To each well were added CHCA (7 µL, 0.4% solution in acetonitrile/water (1:1)) and then acetonitrile/ water (7 µL, 1:1 with 0.1% trifluoroacetic acid (v/v)). A 2.5 µL volume of the mixture solution was

taken up to be spotted onto a 384-well MALDI plate, which was allowed to stand for 15 min to dry naturally. MS and MS/MS acquisition was conducted with ultrafleXtreme™ MALDI-TOF/TOF mass spectrometer from Bruker Daltonics.

Synthesis of individual peptides. Each peptide was synthesized on Rink amide resins (0.63 mmol/g) on a typical resin scale of 20 mg per sequence in bulk for affinity measurements. With the desired sequence of peptide attained, the resin was treated in trifluoroacetic acid (95%), water (2.5%), and triisopropylsilane (2.5%) for 2 h. The cleavage cocktail was concentrated in a continuous flow of nitrogen, and the crude peptides were precipitated in diethyl ether. The resulting white solid was then purified to >95% in purity by HPLC bearing a C_{18} reversed-phase preparative column. The purified peptides were used for affinity measurements via surface plasmon resonance (SPR) and confocal microscope image.

Affinity measurements. Affinity measurements were carried out on a Biacore T100 instrument (GE Healthcare). The CM5 sensor chip was used for all measurements. The sensor was primed with HBS-EP+ (10mM HEPES pH 7.4, 150mM NaCl, 3.4mM EDTA, 0.005% P20, GE Healthcare) buffer and was activated using amine coupling through 1:1 mixture of 0.4M EDC and 0.1M NHS and remaining activated groups was blocked with 1M solution of ethanolamine (pH 8.5). *h*CAII was immobilized onto the sensor chip surface by approximately 6500 response units (RU). A blank flow cell (no immobilized protein) was used as a reference to subtract nonspecific binding, drift, and the bulk refractive index. Next, varying concentrations of peptide (3-50 μM) were passed over the chip for 25 min at a flow rate of 50 μL/min. Association (k_a) and dissociation (k_d) rate constants were calculated with a two state binding model using Biacore evaluation software, and KD values were calculated from the ratio of k_d to k_a. Kinetic parameters were obtained by fitting curves to a two state binding model with baseline correction.

Fluorescence and confocal microscopy Samples were examined using a Zeiss LSM DUO laser scanning microscope (Carl Zeiss, Oberkochen, Germany). The digital photographs were processed with LSM Image Brower software. After excited by the Helium-Neon laser line with the wavelength of 633 nm, fluorescence signals longer than 650 nm were acquired to examine the red signal intensities.

References

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3. S. S. Lee, J. Lim, S. Tan, J. Cha, S. Y. Yeo, H. D. Agnew and J. R. Heath. *Anal. Chem.*, **2010**, 82, 672.
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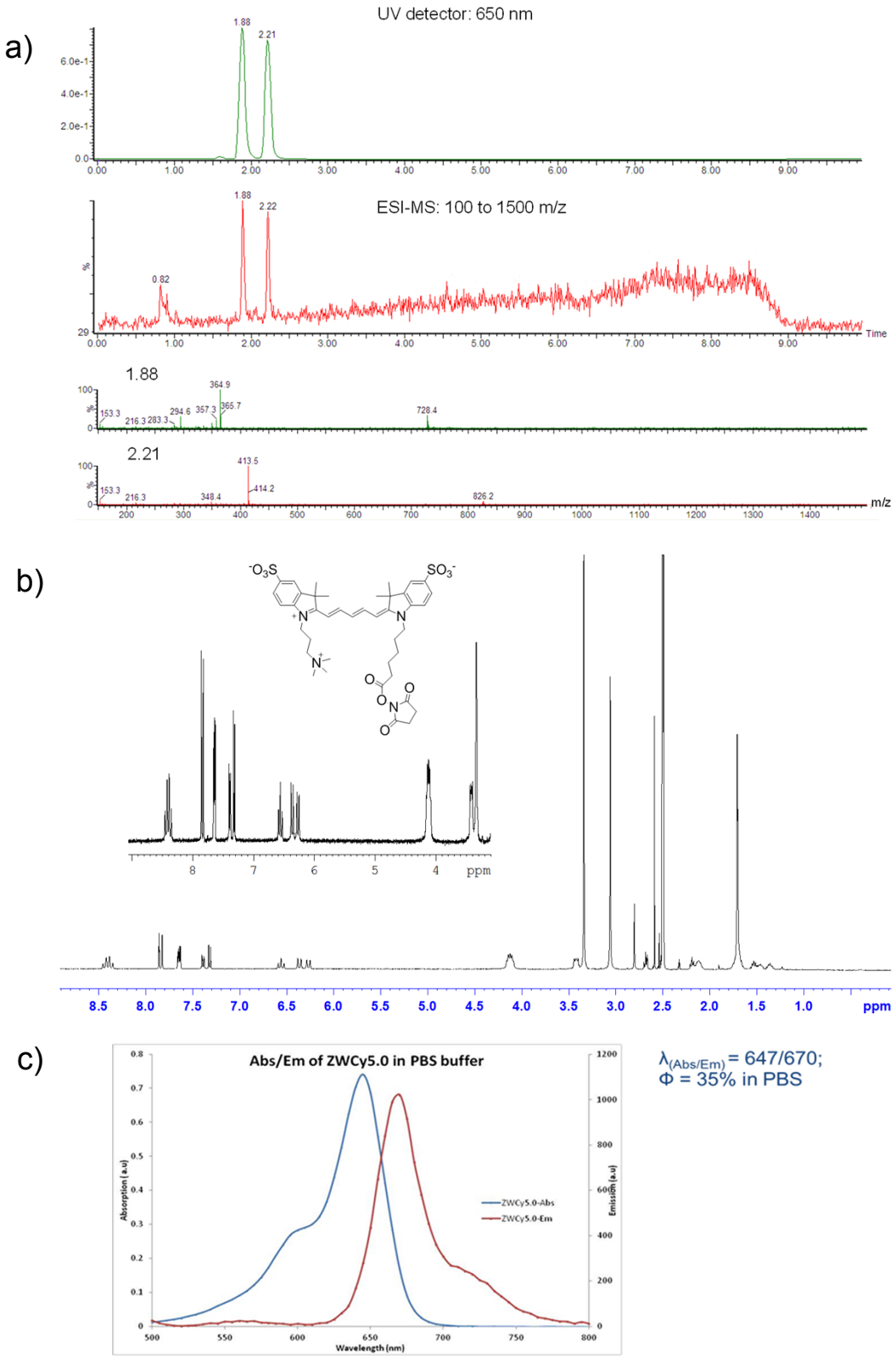

Fig S1. Characterization of **ZWCy5**. a) LC-MS data of **NHS-ZWCy5** (1.88: **ZWCy5**, 2.21: **NHS-ZWCy5**, Some of **NHS-ZWCy5** were hydrolyzed during LC-MS analysis under aqueous conditions), b) ^{1}H NMR spectrum of **NHS-ZWCy5** (DMSO-d_6) and c) absorption/emission spectra of **ZWCy5**

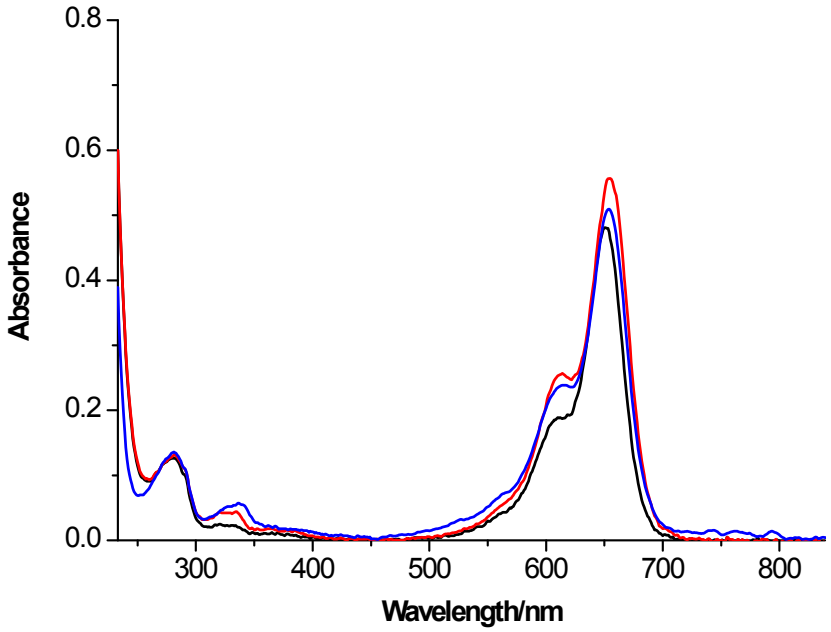

Fig S2. UV/vis spectra of hCAII conjugated with **ZW** (black), **DY** (red) and **AL** (blue)

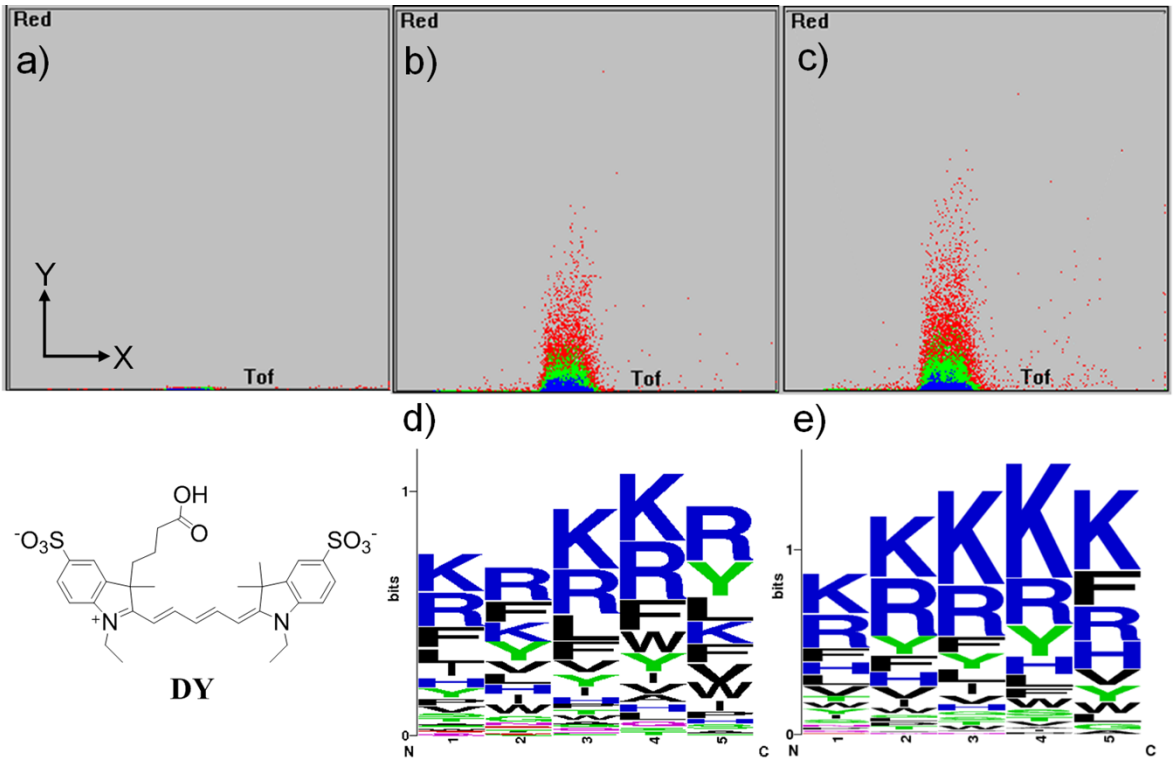

Fig S3. COPAS sorting images (X-axis: TOF; Y-axis: fluorescence intensity a.u.) from a) a 5-mer comprehensive peptide library itself, and its screening against b) **DY**-conjugated lysine c) **DY**-conjugated *h*CAII ([dye/protein] = 100 nM, Excitation: 640 nm solid laser; Detection conditions: Gain = 3, PMT = 750); Font histograms from positive peptides for d) **DY**-conjugated lysine and e) **DY**-conjugated *h*CAII.

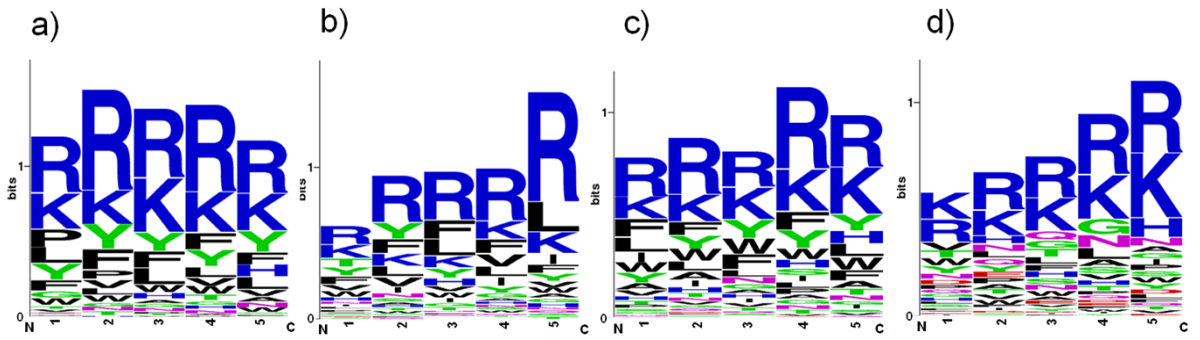

Fig S4. Histogram of positive peptides from screening of a 5-mer comprehensive library against AL-labeled (a) *b*CAII (b) CRP (c) AFP (d) PSA.

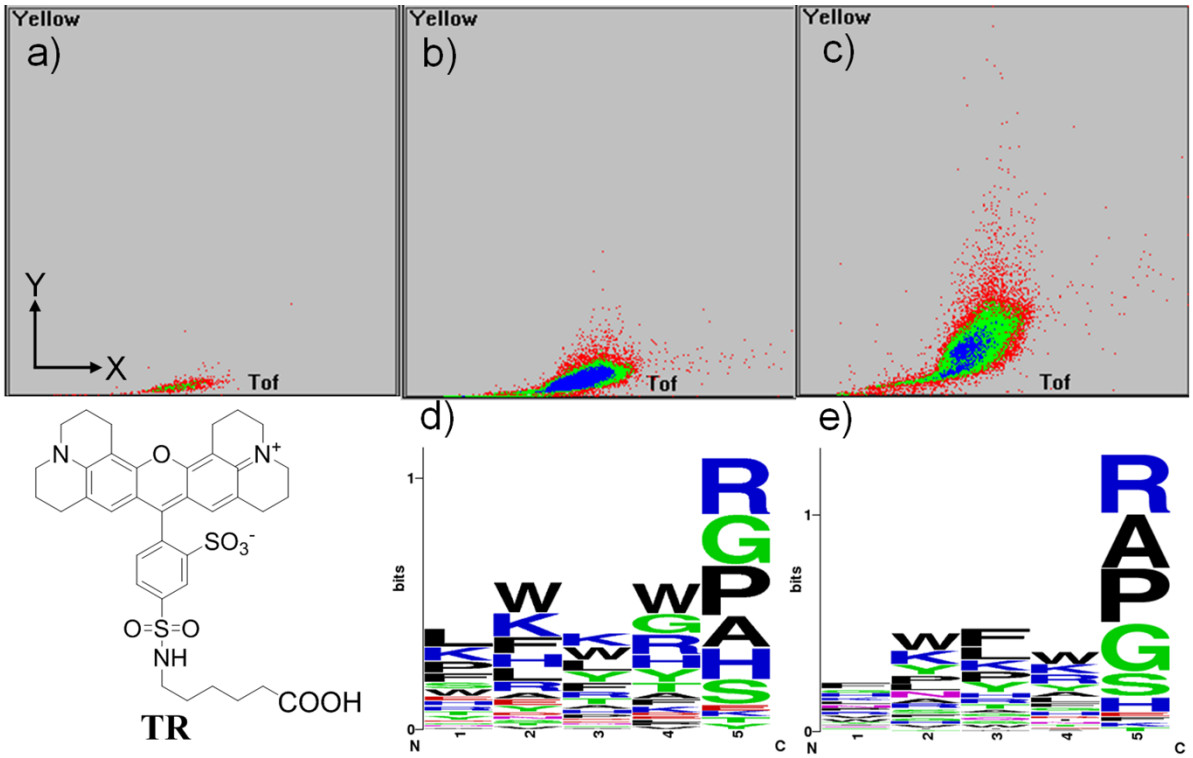

Fig S5. COPAS sorting images (X-axis: TOF; Y-axis: fluorescence intensity a.u.) from a) a 5-mer comprehensive peptide library itself, and its screening against b) **TR**-conjugated lysine c) **TR**-conjugated *h*CAII ([dye/protein] = 200 nM; Excitation: 560 nm solid laser; Detection conditions: Gain = 2, PMT = 650); Font histograms from positive peptides for d) **TR**-conjugated lysine and e) **TR**-conjugated *h*CAII.

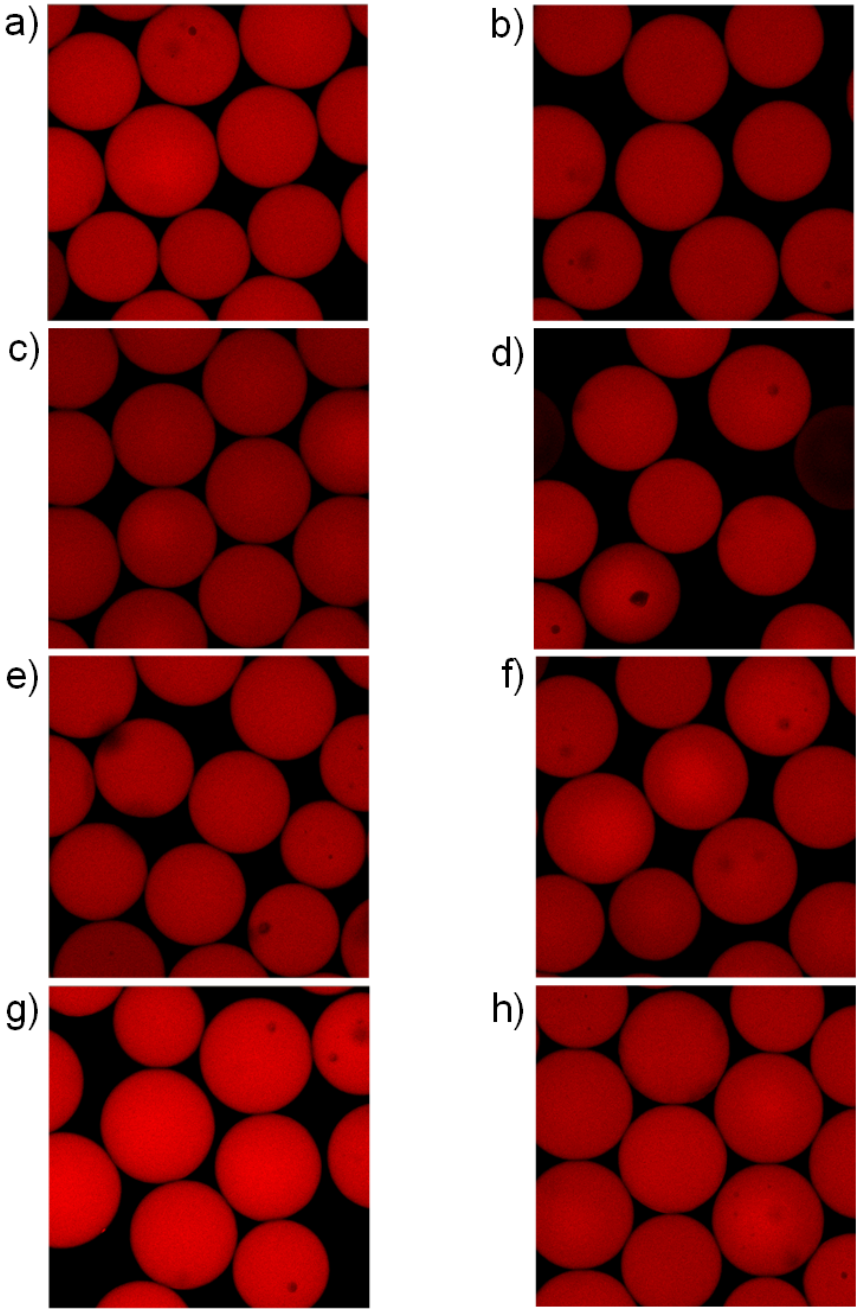

Fig S6. Confocal images of a) and b) hykwr-conjugated beads, c) and d) kpvwG-conjugated beads, e) and f) wnffh-conjuagted beads, g) and f) fvfra-conjugated beads after incubation with **TR**-conjugated lysine (a, c, e and g) and **TR**-conjugated *h*CAII (b, d, g and h).

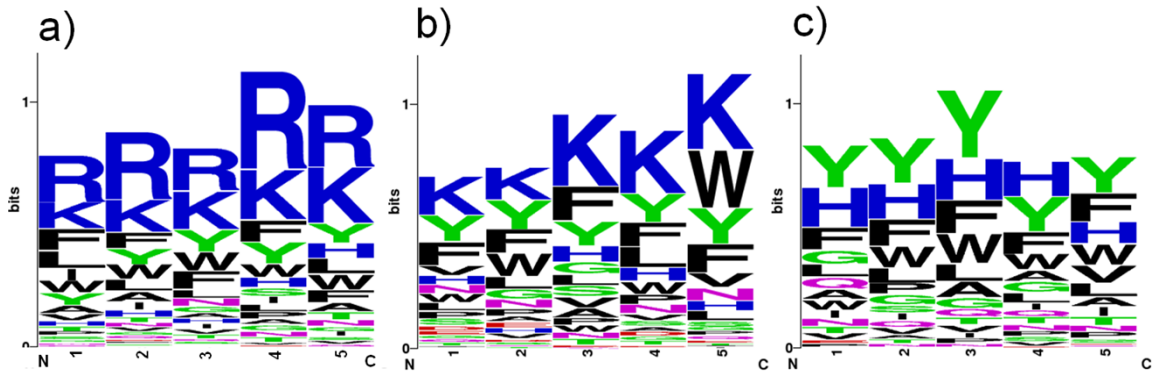

Fig S7. Histogram of positive peptides from screening of 5-mer comprehensive peptide libraries with a) 18 D-AA's b) 17 D-AA's (excepting arginine) c) 16 D-AA's (excepting both arginine and lysine) against AL-labeled AFP.

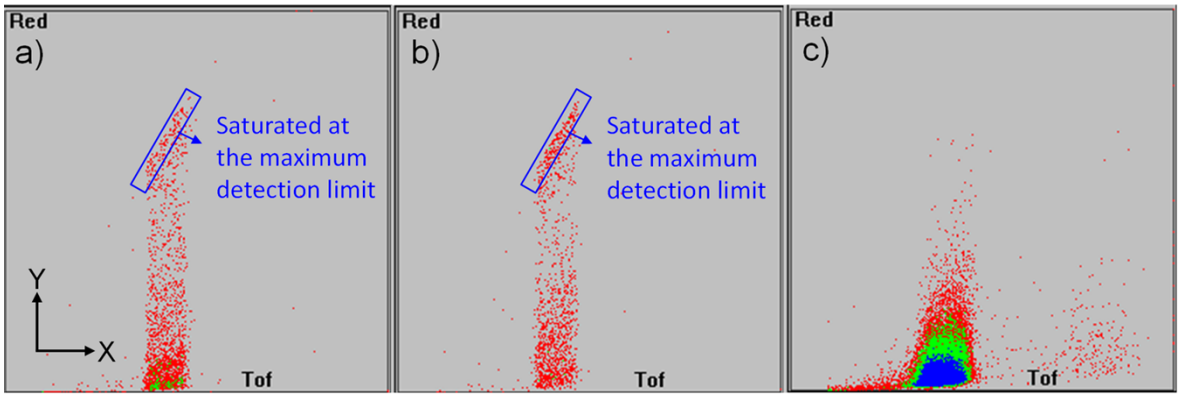

Fig S8. COPAS sorting images (X-axis: TOF; Y-axis: fluorescence intensity a.u.) from screening of a 5-mer comprehensive peptide library against a) **AL**-conjugated *h*CAII, b) **DY**-conjugated *h*CAII c) **ZW**-conjugated *h*CAII ([protein] = 100 nM; Excitation: 640 nm solid laser; Detection conditions: Gain = 3, PMT = 750); The protein concentration for screening was adjusted to give the same maximum emission level.

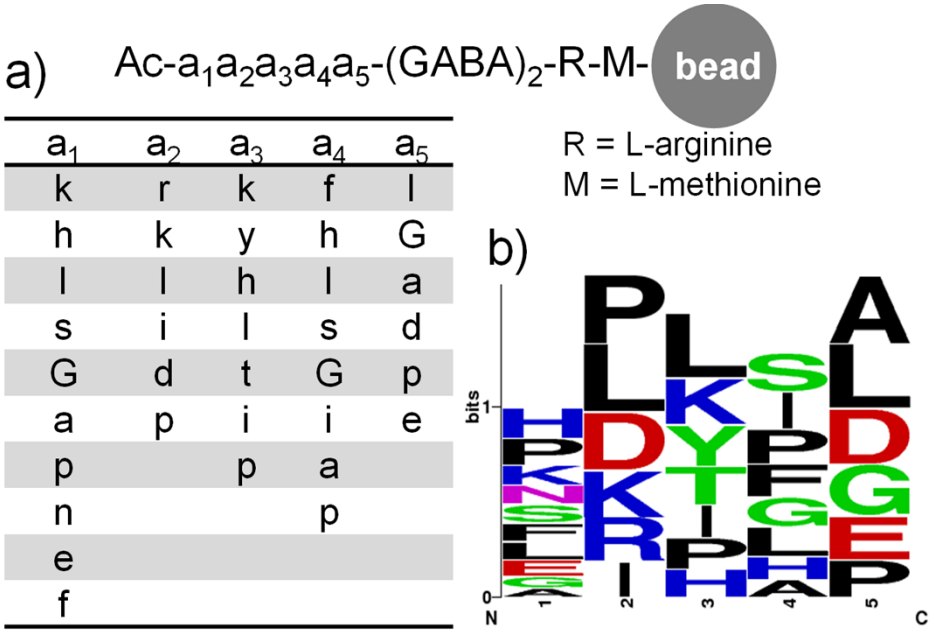

Fig S9. Generation of a focused library: a) The structure of a focused library and b) font histogram from screening results

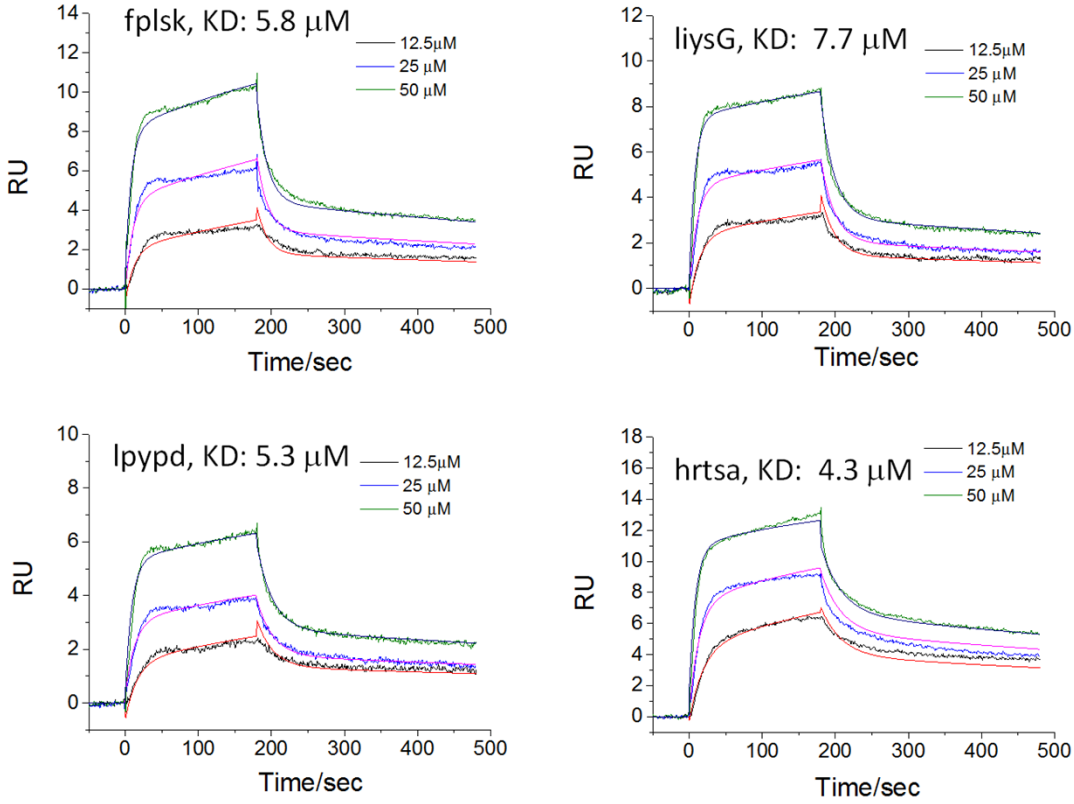

Fig S10. SPR sensorgrams of binding peptides from the screening against **ZW**-conjugated *h*CAII. KD values were obtained by two-state model fitting found in Biacore T100 evaluation software.

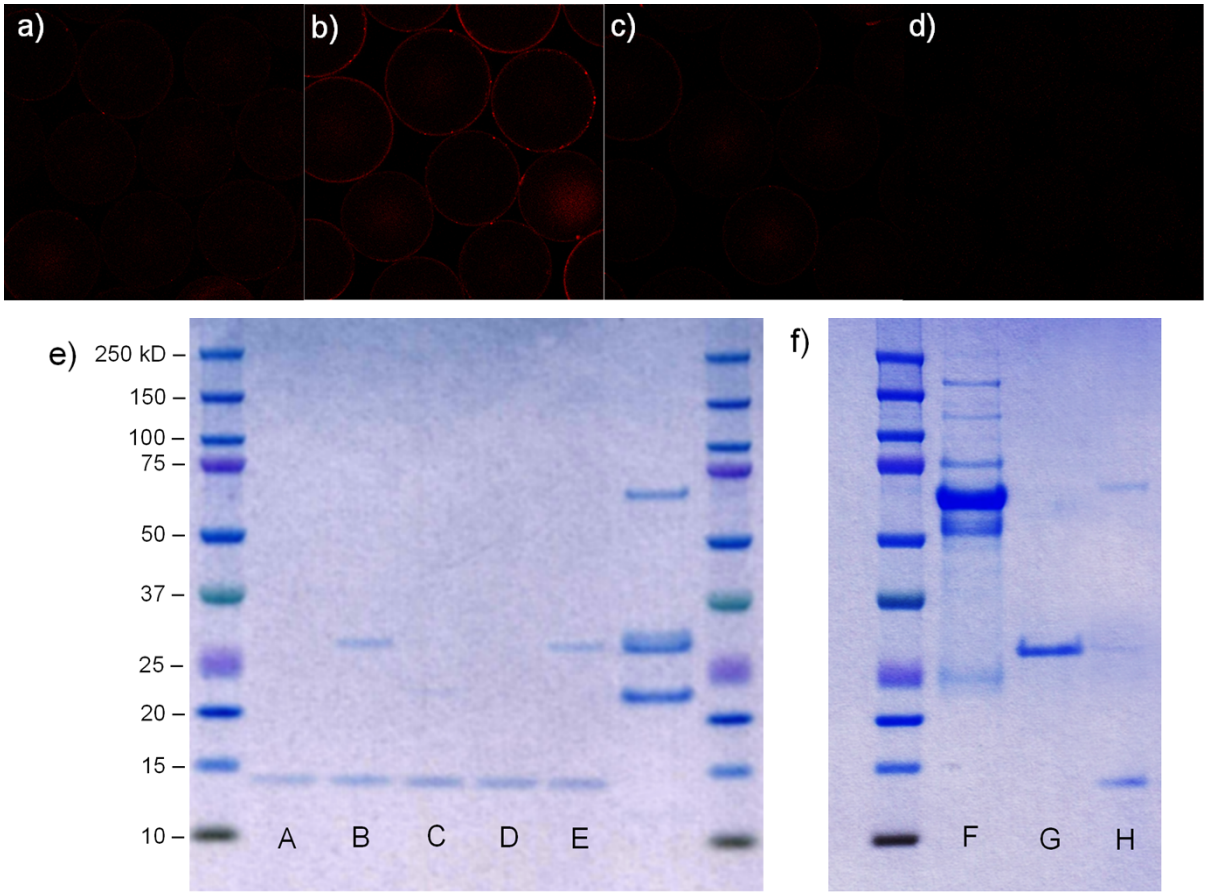

Fig S11. Confocal images of Ac-fplsk-conjugated beads after incubation with a) **ZW**-conjugated PSA, b) **ZW**-conjugated *h*CAII, c) **ZW**-conjugated CRP and d) **ZW**-conjugated AFP. The images were obtained under the same acquisition parameters. e) Pull-down experiment using streptavidin-agarose beads conjugated with biotinylated fplsk in presence of (A) PSA (30 kD), (B) *h*CAII (29 kD), (C) CRP (120/5 kD), (D) AFP (70 kD) and (F) the mixture of PSA, *h*CAII, CRP and AFP. f) Pull-down experiment using streptavidin-agarose beads conjugated with biotinylated fplsk in presence of (F) human serum, (G) *h*CAII and (H) *h*CAII in 2% human serum. Monomeric streptavidin = 13.2 kD.

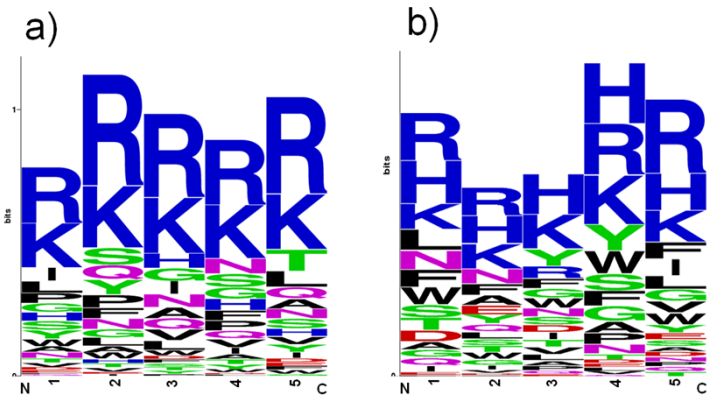

Fig S12. Histogram of positive peptides from screening of a 5-mer comprehensive peptide library against a) **AL**-conjugated Ki-67 and b) **ZW**-conjugated Ki-67

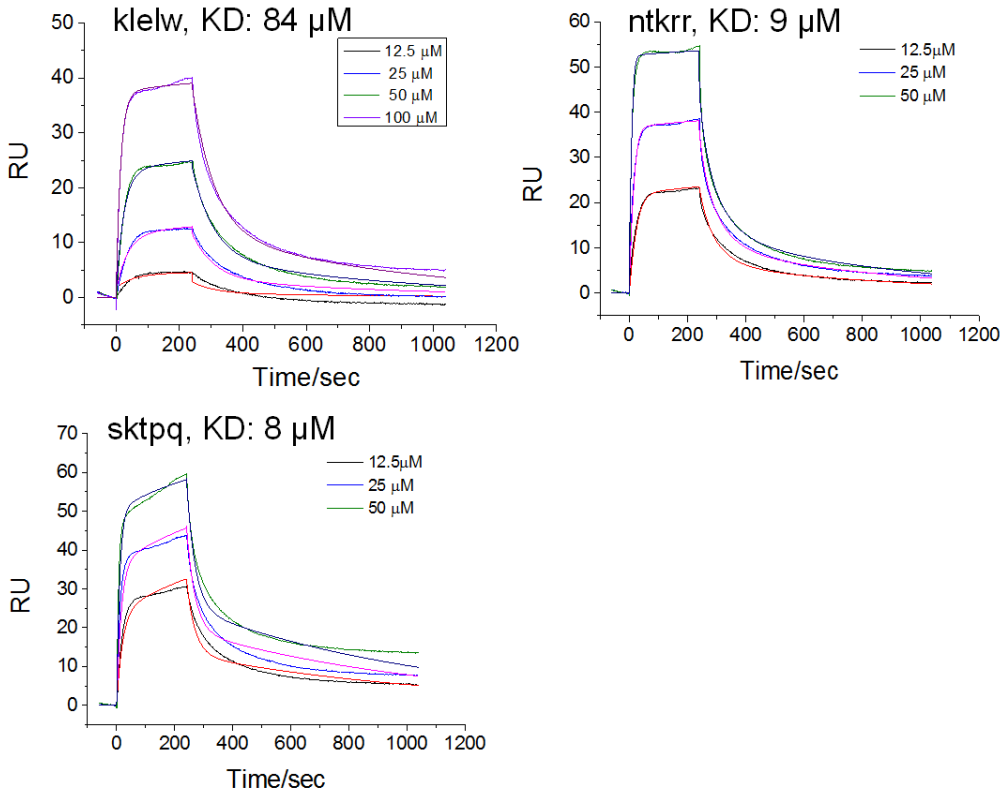

Fig S13. SPR sensorgrams of binding peptides from the screening against Ki-67. KD values were obtained by two-state model fitting found in Biacore T100 evaluation software.

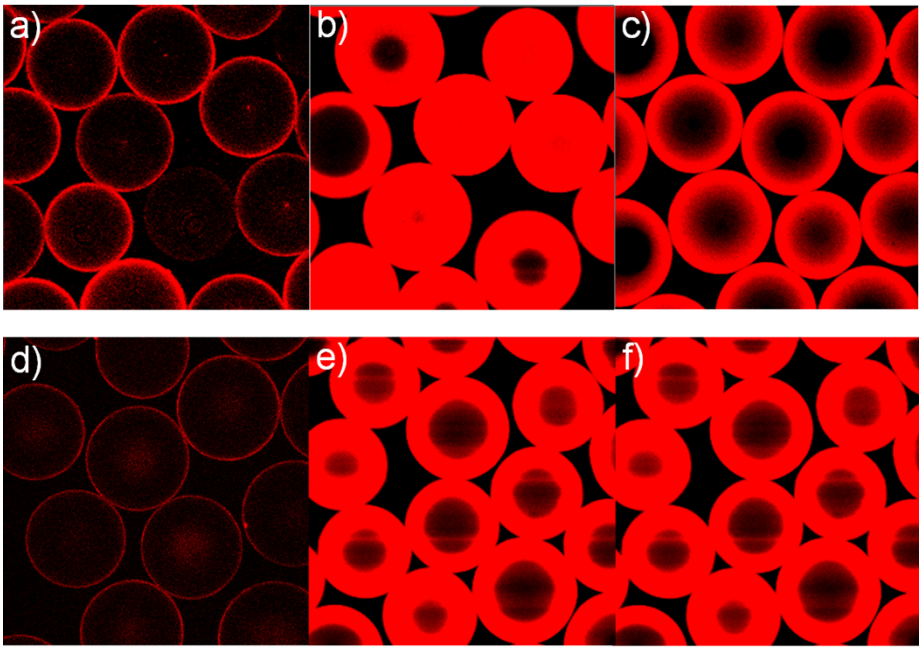

Fig S14. Confocal microscope images of Ac-lklwfk-conjugated beads incubated with a) **ZW**-conjugated *h*CAII, b) **AL**- conjugated *h*CAII and c) **DY**- conjugated *h*CAII, and Ac-lhrywf-conjugated beads incubated with d) **ZW**-conjugated *h*CAII, e) **AL**-conjugated *h*CAII and f) **DY**- conjugated *h*CAII.

Table S1. Positive peptides from the screening of the 5-mer comprehensive library against **ZW**-conjugated *h*CAII.

aaype	eqthd	hdvra	kGnip	lwdsk
afhll	erlnq	hdvwd	kGrsd	nehks
ahlnl	etiyf	hdwal	kGtel	neidr
aihpp	evefr	hfpns	kklql	nekfp
aikaa	evvwa	hhaad	klkhs	nGtwd
aiysp	fdrwl	higap	kndse	nhqes
aiyvp	fhpwh	hlkae	kptsG	niyds
anese	fkdil	hlknG	kqppe	npdll
aprap	fkdil	hnnaG	kreee	npwwd
apwyp	fplsk	hnspG	krthy	npyef
arwad	fqwGG	hrqGa	ktfdt	nsGvi
ayaqp	frdvl	hslpp	ktlGp	nskhl
dafep	fvsln	hytis	kvtik	nyahp
datvG	fwisp	idhpq	leaap	pdsat
dhfey	Gayie	idrek	leisn	pdwGG
dpkrp	Gklek	ilsqp	lkelt	pettn
dtpyp	Glphi	ipphp	lkqfe	pGyvd
ekqlw	GlyfG	isheh	lkvhd	piGfa
eliyp	Gqnip	kalip	llyvi	pnvae
elnqa	GwslG	kdfer	lpfGd	pqhen
epail	Gyave	kdirG	lpiaa	pqpGt
eppeh	Gyisl	kdypq	lqhfn	prnae
eqilG	Gyple	kGlGp	lrrne	ptadG
qahsk	rrrfn	sskGw	trwyp	wstdn
qfken	ryien	stkfd	tsfrp	yaGia
qkkve	sadla	swapp	valhp	ydihd
qvyGl	sekla	swGep	vewGG	yessl
qwtel	shyfe	swpkp	vGqif	yinfp
rahGn	sivae	syaGr	vkver	yitaq
rdlar	siypr	tkkal	vlGsi	ytywd
reGhq	sksyi	tlihr	vslll	ywyaG
rGehh	spiwa	tpnld	vsyda	yydfe
rifrq	sqpps	tpyet	wGipp	tyw
rpiys	srhen	trryp	wlite	

Table S2. Positive peptides from the screening of the 5-mer comprehensive library against **ZW**-conjugated lysine.

aaypy	aheyd	ndhye	yykkh	hdeyy
ltvvy	ddshy	dqdhy	kGyrh	dylhd
vvppl	hsddy	ndyeh	hdydh	ydhve
Gnkyy	yhykv	hdykd	yhksi	yhidd
hnsda	dhysy	veyhe	yGwsh	nhydd
kdGdi	yyyhd	ydyhy	dyheq	htdey
hsdtp	hihyd	eyhdy	dyheG	htdye
kGykk	yehae	yqhde	hdady	heype
enliy	haeye	neeyh	gdeyh	hyyde
hdyvd	eyhea	edqyh	ehyGd	ysywy
hhley	akyky	yqhde	kkhhh	deyyh
hqave	hsydy	eehyh	lthfh	yGysr
hdsdi	hseyd	nheey	ykyki	dkdyh
hesyy	eyhsd	dqhye	kkyil	hddyy
sehyy	hydtd	dqyhe	ydhae	dhqdy
dhlse	ehdyh	rkikr	yahed	yhdyd
svrnk	sheyd	kkkyh	yhryh	edhyy
hvydh	iyhgd	ehhye	kwkkr	hdeyy
hvsfd	diyvy	hedyy	ykyyh	hyeey
Gyhdd	diyvy	yyedh	dyhde	yrkky
yfGtr	hhsyd	dhyey		
hsyyp	kqhkh			

Table S3. Positive peptides from the screening of the 5-mer focused library against **ZW**-conjugated *h*CAII.

adkfe	eplpl	Gihla	hltfd	kiype
adtil	erkse	Giple	hphfa	kkial
akisd	erkld	GithG	hphlG	kklie
alifl	erphl	Gkpsp	hpifd	kllal
altld	fdkia	GliGG	hpksa	klpll
alysG	fdplp	GlkaG	hplhl	klyGp
apkfp	filfl	GlkGd	hplld	kphpe
arlia	fiyla	GpilG	hplsG	kpiad
artGl	fkhia	Gptie	hptap	kpild
ediGl	fkkpe	Grksl	hptfa	kplGa
edlaG	fkliG	Grtpl	hptGa	kpplp
edpfa	fktgp	hdlGd	hptlp	krpil
edpsp	fkyll	hdpfp	hpyia	krysd
eiyhl	fkypd	hdtpG	hriha	ldhsa
ekhsG	fllap	hikpG	hrkGd	ldipG
ekisl	fplip	hitiG	hrkpl	ldkGd
eklfd	fplsa	hkhpd	hrtGa	ldyse
ektle	fppGl	hkifG	hrtsa	lilfd
elkid	frhhd	hkkid	kdhpG	lilsd
ellGe	frisa	hlihl	kdkGd	liyfl
elpia	frpid	hlkse	kilaG	liysG
elysG	frppl	hllfl	kilfG	lkppa
ephGG	Gdhha	hlliG	kilGp	lktil
lllsp	nlihG	pdyGe	pplpp	slill
llpia	nllGl	pikGp	pppia	sllGa
llyie	nllie	piyla	ppypl	sllse
lpypd	nllla	pklha	prkGd	spihd
lpype	nllsl	pklil	prpia	splpe
lrthp	nltaa	pkyie	sdhGp	srhaG
lrypp	npipe	plise	sdlGl	srkid
ndhlG	npyll	plkfl	sdlhd	sryfa
ndksd	nrlfG	plksa	sdtfd	nlah
ndlsl	nrlpl	pllil	sdthd	nlhal
ndtsa	pdkha	plphe	sdyha	pdtaG
nihpe	pdkpG	pltfa	sikpp	pdtfe
nkhfd	pdlfa	ppkaa	siypp	ppksd
nkpla	pdplp	ppkfl	skhhG	ppksG
skyie	slila			

Table S4. Positive peptides from the screening of the 5-mer comprehensive library against **AL**-conjugated lysine.

dshwh	prhng	hhkhn	rwgwk	gpkrl
nnrry	rgkyr	rkryt	gplfn	rgrwy
evgre	rkkyi	ayyhs	wgfnr	ykrri
vdldl	khrrf	qkfrk	kkdfg	kfakk
vwfrq	sgaad	pyeke	ryrha	frrlk
sgyfr	hhrrw	grkrl	rkllr	ratka
hlrkl	pfrry	vkkpt	idrfn	yagpt
grvfe	kqksr	rpkyk	shrrk	ryqph
klsri	kehwp	hryrq	lkrfk	gskkr
kkdfg	slllk	glval	qkfrk	rkkyt
hpkkn	akrfk	aavle	rrkys	krlkr
vkkns	shprk	hprrw	kirky	rygkk
rrwry	ggkre	aprrw	hlrkl	lvptw
lkikv	rryrv	hsskr	gptkv	ltkty
svvvy	rrrfv	slllk	kgrpv	pegnl
qnetf	sknlr	vlfnp	rlivl	rikhr
qkhry	epylr	rpytv	srhvn	arash
khvrr	ldlki	kwnfr	rfakf	iflrv
rdhav	kprra	hpkkn	plhal	gseyw
fvnkr	pyprl	rywka	raskr	kihll
yrrtr	grrwv	twrrk	trfrl	qrlrk
lqlkv	rffkk	krtri	lrrgk	wnfhs
skafn	rrrfv	rleyr	rflyv	hrlry
tnhrr	arrlk	kykrs	vrqkf	gvyqi
iykrk	rlieg	eghwa	ranqy	vhrrf
kkrlk	rtkrk	rgwla	gqgnw	wgfgk
dshwh	nnrry	evgre	frgkf	

Table S5. Positive peptides from the screening of the 5-mer comprehensive library against **AL**-conjugated *h*CAII.

rikrh	ylrkG	ykrkv	hrkfy	ryphr
krwqk	vivvk	kfkkv	wkrtk	vkvqw
kfklr	frfkr	lvvkv	hfkrG	vvfyr
rffkk	krtry	fkfpr	kkkkw	yrikk
prwkk	hrkka	qykkr	wrkhk	sikvv
qrrkk	kkhfw	frnhk	yrhrk	lkGkk
irwkr	kirkk	pwkry	krhvr	wwkfk
kyrlf	kewly	wkrtk	wrllk	wkwlk
hhkar	wkfkh	lrrki	rrwlk	kyykh
kwkkf	kfrhw	ffkrk	vflkv	Gwrkk
rfkkw	tnhkr	rllkk	hqrkh	tryyr
wkirr	fwkyr	phkrv	Gkyyk	lrkkf
fvkkk	ykhyr	kyksh	prykr	lrkyk
rhkhk	aklrk	iwkrr	vlvki	rhykr
klrkw	wfhrk	trwrn	rvwkr	yhrkk
Gkfkk	fyrkk	rGhkk	kkyik	kffhk
pykrk	rfrsk	fkkvy	ykrkw	hkkff
kkkqy	frkra	iwrrk	yrkkk	rllkk
ryyrh	irkky	khkry	wykfk	yrrkr
kwkkl	frkpk	frhrr	wrkry	fklvv
vlivk	krkyl	qhkrf	rkhGk	rhkvk
fhktk	ykfkh	rrkwf	kkvrl	hhvkk
krkfi	qrwkk	iyvkv	rwfkh	kykry
lrrky	kwfrr	Gyrhk	wfkkk	karyh
kfkkh	flkrk	yrnrk	kwkrw	kkkly
hrvfk	fkkyw	krnqw	kilvw	nflkk
rqhry	hlrrk	qykrk	rhwkf	ykahr
fryky	kkhff	rlkrw	wrkhr	vvryr
kqykk	hkyrl	rktlk	rlykk	hrkhy
vkkqr	yGkrk	rykyv	skkfk	wvrkr
hkkrf	khlqk	wrkry	hnyrk	kyhhr
vfvki	kkyyk	hqkkw	irkhy	vhfkr
rirkf	hkkyw	ykfkr	trfrf	
kkrkv	fiyvr	rrhfk	srkrf	

Table S6. Positive peptides from the screening of the 5-mer comprehensive library against **DY**-conjugated lysine.

itkrf	gnnkl	tfrfk	lrlkv	gaprl
lhfyr	klriy	lrktl	iykkr	sghrf
fgrry	skkqs	wklir	pykyk	idfvr
hfrkr	fyvql	rhyhf	ivkfr	wvfyr
rvykk	hfkry	kyfiy	frraw	vrrwl
ryprw	frkyy	frkkk	fkkwl	vlrkv
rlrky	kwvry	nyrfr	ryrkw	yrkrr
rgqrp	lrlav	riikl	hfywk	fkkkv
khikl	krarw	krkkr	ivkkr	kfrsr
ryvkw	kykrr	rfhvi	kwkkv	likfi
kklky	kkgfv	yhfkf	yvrky	rrtsk
rriyr	kklfv	arlwf	lrlwy	lvvvf
aikfr	rhfkw	fnyrr	kiilw	kfyfk
rikrf	fkkks	krrhk	tfkrr	klrvk
yvryr	rirky	irfil	kwvfr	pshrw
iyrrh	lrtky	rfkll	pklrw	ywkfv
kirfr	krrwi	flrra	knkvl	vkyaG
hfllr	flwhr	fwfrf	rytry	rkkyv
rrvfy	htwry	prkyr	khgra	hrktk
kflkk	ryvky	ifafr	egkwp	krlhs
fvrwr	rwkyy	veraf	veqrp	kkpri
hlylr	qfrkf	lwkrr	yrfrh	rvlil
kylkv	lklki	frfri	pkskl	ihwwr
ylhkr	rykwy	rgikp	tfrfk	sfkqh

Table S7. Positive peptides from the screening of the 5-mer comprehensive library against **DY**-conjugated *h*CAII.

lkrkk	fvlkw	rrkwk	yrrfh	rrkyf
lrrkv	kryhy	frikh	trhwr	irfkk
rrkkG	nkkhy	kkrlf	nykhr	hhkkh
krkkv	rfrlh	pkykr	rklkf	lrfrk
kykka	fykkl	vryhk	fkvkk	qysyk
rilrk	hklkk	knhyk	kfkyh	rfkkr
rkkfl	shkrw	kkGhr	rrrkf	rlryk
trrly	vktkh	yrrkG	skykr	kifkk
kpklf	wrkyk	rtkkk	krrky	fkryh
kwrrf	skkhy	ftrrk	frktk	kfkah
ihGhk	trkfr	rkkkf	rwrkk	vfkkk
kpklf	rrryh	lkrkf	hfrrk	lhyrv
wkwrk	hkssk	rikrk	kvvff	kGkkf
vykkf	lrfkr	vkrsw	fkkka	wkkrk
kfkkl	vhkrG	krakh	kvlky	rktkh
vfvrv	kilvf	rkhik	kkfkw	frykf
hyfkr	qakkh	pyryk	dviwr	kykrk
lhiwr	wkirk	hykyr	yknkk	thkkr
yryrr	rkkpk	kkkvl	hGkrk	krvkv
rrikv	irwrv	kwrrk	rvfky	klktw
rkkyf	hkrsr	krryk	rvkkk	

Table S8. Positive peptides from the screening of the 5-mer comprehensive library against **AL**-conjugated *b*CAII.

kGrry	pprkf	rrrrn	fkrkh	yfvkr
Rfrkw	pryfr	rfrrf	pyrrr	yrrwr
rrkrv	frwan	lrtrr	klkyy	rryrl
kkrkk	krrfa	rrklr	Gyrrr	kyknr
pkrrw	lkrrh	krkhr	trrkh	rkGrl
wyrrr	rwkkG	wtrtr	krwrk	srhhr
Grrry	frnrf	lrGkr	yrrfh	wrrlv
trkrr	rfkpk	rkkkf	tykvr	rkary
kwkyr	rrlvn	krwkk	rpnlr	arkty
yrrkw	prlrr	krhrh	rvkrh	kfkyk
kykrl	vykkk	kryws	qrffk	rffrl
rkyrl	lfrrk	yryrk	pwkyk	kvkyf
rfkfk	rrkyp	fyrGk	kklfy	Gyykk
krlry	lkrrr	pvlrk	wrlry	krfkk
ykrnv	vrrrf	rGklk	pvrGy	vnfvr
krfyr	rykkv	frlry	lnlrr	fryrr
rrrla	lhkrr	lvrkk	prawr	yrfrk
rkpry	rkfkr	lfrkf	rrfkr	rryvk
Gpkry	rpkwr	yrrfa	rryyl	klrfk
pkwkr	rkrrr	rrhkr	yrkrk	ryrrl
klkrh	rrftk			

Table S9. Positive peptides from the screening of the 5-mer comprehensive library against **AL**-conjugated CRP.

krfny	krir	yvryr	lfyfr	kanrr
vkrrf	tfyfr	rytqr	frwvl	krrnh
yrrrq	rtrka	kflil	arrvr	srrkw
rtrrw	ksprr	wlvrl	vrfyl	fifrv
fvrvr	fvlfk	nrrsk	yvvvr	yvylr
rrrtq	kfhrr	kykkr	yillh	hrwyk
ksrrr	lkrrs	ifkrr	vrlvr	pyarr
rfyly	hfill	wvrvr	wrlyl	rrriw
fkkkr	tnrrr	ntrrr	wrrrn	rrfsa
yrivf	irqir	khkkr	krrkg	prkkv
rlfif	wyfrv	v ylrw	wrfqi	tlwkr
rfwfr	kykky	erryr	karrl	rwvvr
fkrrs	hylfl	wrllt	tnqrr	vyfri
fllvr	rigkr	pkgkr	hyrrk	rfhfi
tirkr	rllvr	rrtfk	rrfak	wkvrl
ryrra	krvkr	yyfrl	hkrlr	wrtgr
rfhfr	rrhfr	fvlfr	tvrar	tyyrr
ffrvy	krrhl	ksyrr	wrdrr	fiyvr
erwrr	rykak	tnpkr	nkrnr	lrlll
fhvrl	rgrhn	nrrrl	rftvf	ylirl
krhgr	arrrg	wqwn	yrffl	rqgrk
tflii	frkri	rkykg	tfrir	yifrl
tkrri	tpwrr	rrrr	tlrrh	yliwk
iqrrr	vlrir	yfrlv	villr	qyirr
hrrrt	rrsik	ikyrr	rggkr	tkrfk
krflr	krnwr	krkpk	arrrl	srrri
rkyrr	hyrrf	grfgr	kwhgr	ylffk
vlffr	vrfry	rrlgr	fnkrr	ryhkl
rsfrr	lrhsr	nprtr	vrlif	
grrer	gykyr	qhrhr	kakyr	
rnrwr	pwrrk	yvlrl	kvfkr	

Table S10. Positive peptides from the screening of the 5-mer comprehensive library against AL-conjugated AFP.

irktr	rknyk	nwkyk	lrlkl	riqrl
lrlsk	yqrky	rsftf	vlrkw	wkrwq
kkyk	ftkrk	wafky	trwfr	irfyr
arlkr	kdrrl	varrr	rhfkt	iiwak
rlrka	ylyhk	rwwrr	Gkfyk	rtysy
irkrn	rfGrn	ykkhr	kkykG	hwrpr
fkykk	rwvkr	hwykr	krtky	rayry
rrrfv	alrfr	rftrl	wnrkf	ywyyk
ftGrk	pkyry	srwrG	rwqpk	fhwhk
kdnrl	kkwrk	sylrr	kafrw	afwpk
lriin	vhrrf	wqirG	lkfyk	flwsr
kklGr	krrrh	kwwhr	rkprk	yykfk
ralsr	trkrr	ferkr	fynrs	fikrh
Grrkf	yrnkk	krfGk	nlwkr	hwhkl
arhrh	kknrk	lrrGw	arryr	rrsrl
vrkpr	fnrry	ylkra	pvrky	qiirk
wrkGk	kGykr	firik	vykpr	sfwrh
rkfki	lrksf	rlynl	rikrh	fkffh
rffak	trkrl	wrGrk	kryrw	lrhrk
vktrr	rfkys	rfnkw	kGkrq	wralk
rhkrf	lrhrh	fysfl	pvrky	kwnrv
rfara	rwylk	irirt	rtGrr	kkrwk
arfrr	wrkkk	tfadw	rpwhk	ihkrr
prfrr	lklyr	kffwr	itrwr	kkGrh
srvlk	lkyry	ryrkt	lkqrh	fvnhr
lkryw	krkyk	irfrf	wrkyk	fytrf
arffG	lrkkr	rkikh	irary	rrksw
lnkkr	wkyri	ravfw	yklwk	rryfh
kavfr	afrar	iryia	rrllk	hwkri
lryvk	ylfra	rsriw	fykrw	irriy
rrrkl	kywhr	rvwkl	frrr	Gpkww
kryah	rrkkn	tkkwr	rfawr	irwvy
fkykk	rykrr	fkyrt	kaknk	vretr
lrrlk	qrkfa	wyrtk	lrkrr	yrwrt
yrrfs	fyhrr	hwykr	yywri	rfwkn
rrrfv	rlGfy	rrrfa	wqfrt	rrryr

klvfr	frrnk	rGsfy	karwf	

Table S11. Positive peptides from the screening of the 5-mer comprehensive library against **AL**-conjugated PSA.

arskr	ikfnk	kvlGr	rklrp	vkrkr
dekGh	ikGkk	kwrrG	rnrar	vkwhd
dekGh	kawnp	kyikr	rqkir	vnskh
dekGh	khdpw	kywkn	rqsrr	vrrsk
deqGh	khqkr	lardk	rrqkt	vslkw
deqGh	khvkr	lardk	rrrtl	vstph
deqGh	kkerp	lrrri	rtfrr	vtGlG
dfkrr	kklGr	lwtes	rvhrf	waGvk
drskk	kklhs	nirhk	rvrsk	wfrrr
ehilh	kklpk	nkekd	ryfnk	wifek
eiwap	kkrqG	nqekd	ryhkr	wkklp
ewssn	kkvrk	nrGrr	ryvkr	wkyrk
fkkkk	klrrn	nrrtk	shkGh	wnnfk
fqkkr	krakr	phssi	skvkr	wpkrr
Ghkkk	krffr	pkrry	tidwr	wpnfk
Gkkna	krGkl	pkslr	tlhnw	wqane
Glpha	krgkl	prnrk	tnqnr	wrryk
grftd	krkkr	pryrk	tnrqr	ykGrk
Grplk	krkrf	qkrrr	tqrkr	yknrw
Grqav	krrrs	qrklk	ttraG	ykrrs
hannh	krtrk	qrkrr	twfrk	yktir
hhere	krtrk	qrrsr	twkrk	yphai
hkewh	krtrt	qvtGn	twryr	yrrkr
hnrrk	krtrr	rdlli	vewdk	yrrtk
hvrrr	kthnk	rhkrk	vfwGt	ytkeq
hyrrk	ktran	rkklh	vhrrk	epft

Table S12. Positive peptides from the screening of the 5-mer comprehensive library against **TR**-conjugated lysine.

edakr	dwkGh	wfkGG	rhrlp	rwkwk
sefha	wslyG	fywtG	sknwa	sllhG
klawG	Gkyya	nwwyh	pwsGy	lwtwp
yrwah	thdGr	pkeys	lwhra	lftrr
pfpph	kewwp	ekwfp	lvyws	vwwGG
taftr	frfGp	lwkwt	pakrh	ylrka
hwyas	lnpwr	krfeG	fhhfp	ktyhp
ffyrs	lylhG	lktwp	wklrr	kflre
kkvtr	phrdr	hlkvr		

Table S13. Positive peptides from the screening of the 5-mer comprehensive library against **TR**-conjugated *h*CAII.

rapwa	pwpkG	wnffh	naffr	hytaa
whfnG	ffyrp	wwfwa	vwlkr	wnltp
lkfpp	vahka	yvhrs	vifvp	Glfap
ytpep	hptGe	kshdp	fvfra	slqwG
awkhG	trkwr	Gywwr	rsfyp	Gkyar
sllrs	hwkyt	kpsGG	kwrrp	Gwlaa
inphp	lkGwk	rytiG	hykwr	GkGrr
kpvwG	yGphG	qfaGh	lykyr	qwsth
pwikr	fkalr	pklya	lhyia	nywka
rpyrG	nrfna	hrylr	fwlds	flrla
fkqkp	fpkwr	nnpfs	aGlys	pkylG
vsrkf				

Table S14. Positive peptides from the screening of the 5-mer comprehensive library (17 D-AA's except r, m and c) against **AL**-conjugated AFP.

ygffk	wykyk	fkffk	fnkph	kwfhy
yngkf	wwtkv	kwhpl	pgfky	kwklk
kykvy	vyfky	kffkk	npkww	kkfyh
gyqkk	kfkfw	fkkwp	kfshk	dgktl
vykkw	kfkfw	kfypw	ffyky	ghwaf
kypgw	yyfky	klfhf	qkwhf	kwayw
vykkw	yfksy	kvvkw	nanvw	pgfky
ywyak	sefdl	fkgkf	ffkwv	lpkww
yyykk	hkhhf	qpvkw	sskyl	pfkkk
fkkkn	kwvky	ygksk	iwgky	kpyww
daphn	wwkhv	llttv	fkskl	slkyk
lwfd	fwlky	hdygy	fykks	fkkwk
qyykw	sgvpw	hpkyw	kylkn	denpw
kfykw	pkhkw	nkkky	ykysy	kyhhn
fwhkv	kwhvq	hyknv	yklnk	kwlkw
epken	yksfk	hqvln	ylkkl	wfalk
vqfyi	kvfkw	fakwk	skwkk	lvyky
lfkyf	kwflk	kfykt	khhkk	yyflk
agkef	yygkf	lfvkk	newde	ykghw
knkys	tfkfy	fskys	depnw	ntkfk
wyayk	engfw	yltlt	fykkf	vfkkw
pnkkw	skfkp	fykhk	vfsyk	fkpyf
hyanf	fyfwk	kfaly	nhwyk	kwayw
kfsnw	fknlk	wwkhk	vwkfy	dpygp
kgfkk	ygylk	ywyky	tsekk	yffky
fknlk	flkfy	pgkyw	ynlyk	ngslh
ywfak	kfkfw	kykwv	fdyte	kygff
tesdf	vykkw	vapfl	vhywk	fkyly
kllkf	kygpw	vfsyk	lvksf	kypfk
wktlk	gvkfk	kkffw	swadw	awlhg
qyyvh	lvepf	yykgk	llwkg	knkvf
hlffk	kyffh	wkkly	ehlnw	plkyw
yafwk	ykvyn	engpk	kkvyk	kwhff
enhyq	yfykk	nlvlw	nwgnw	wyhyk
lknpq	kfffa	fkhly	plfkk	ykykg
fhkkk	fkklw	gkkty	kffyh	hkhqh

sdann	yeklv	tkfss	hlkfw	yylak
npskn	hakyv	ytkkv	ykhvk	wwtkf

Table S15. Positive peptides from the screening of the 5-mer comprehensive library (16 D-AA's except r, k, m and c) against **AL**-conjugated AFP.

wyhgv	wqgwa	lytff	yhwph	hlqqv
yihyh	hiyyf	qhyyf	hwyal	liyhw
nfyin	hsplf	ayyil	gglhy	ntafl
hwyhv	yhfyi	fhafl	nywiw	hqyyi
awhyt	ypvhy	ylqvw	vhyyy	vywv
whyya	ylhfv	fhtwl	ylpyy	qfyhh
yfysf	hwwlv	hlywy	wlfst	hhgyw
yaflf	gfyaf	vswly	hhyyp	ffyhn
fnynw	qpfhw	qlyya	vqhyy	qftgy
wnysy	yqwhw	npyhf	yvhft	fpwya
hgwny	gylyl	ypfyy	gffvh	asfn
yhyyp	yvwv	ifyhf	lytws	yfhni
hyhhv	fpiss	ehqyy	lhfav	hwywa
flhyf	ahyyp	fghhw	ytwty	afwgy
ltily	fyagl	lpglf	nhyfv	hnhww
gglhy	igayf	hviav	hwhag	efgdd
wsfha	hfyqy	hyafh	gllyf	ypvtw
hhlly	psvtv	iwygh	gsyff	yyyly
wtfaa	iwyhg	yhwqn	qfyfh	hffft
hwypy	awwhi	fygpy	yhayv	fggyf
hfhhy	qighi	hfafl	ahawf	vqyn
esvfp	yiyhn	yfapy	lwhfh	tifit
hfyhf	yffpv	yvfgh	iyqhy	pahqw
yfwhn	ylyyi	vnlnv	lfyvn	qylhw
gqwhi	iyffa	gwwfh	lffpf	thyyp
yghgy	ywaah	qhfyh	hyvwi	hhyyy
hwhwn	gyyhf	hyway	yyhyv	layyh
flyhy	hiqwt	tlwfl	nhfal	hfyly
ywtaa	awqfi	ythhy	hyfll	wgqhh
vhatv	fhyhv	ygfqn	flanh	nhhfl
yiwyh	fwyhf	wwnnt	ghppf	fgpyf
wyhht	fyvgt	alwfp	vgyhw	yyyay
gsfst	fyyqw	yhwqy	yswhf	hllwv

qylhw	qwhaf	hvfhf	fplyw	fyhlv
ewndq	hyhhy	hvfwa	aywgw	wwdta
hwfgt	iyyii	yypfl	ygfly	ylhnf
ghqsh	tyhwf	tvlil	nwytf	hpfyl
hyfgw	iygve	vpyfi	pyhwh	qhyqw
fyhiv	hylfl	fvhyf	fpiyw	fwql
gfhqy	gflwh	fyhhh	hfwfh	hyyhw
qqfvl	yhwhi	hytwy	slhwv	yhyyg
yftyq	tyhyh	ayhnf	tylhy	whysh
ydnwd	ayvtp	fylhh	lhwgw	yqwyw
iayyf	yqyfa	fhaiy	lyhhy	gyyaf
hvlwy	yfwhh	awhwy	hyfiw	lfaGy
npfhw	yygtf	hywah	qhyyv	gfhyy
hshfh	vhyfp	iyylg	thyyv	yhfgf
yafhy	lyifs	pygfy	yhavf	lhytw

Table S16. Positive peptides from the screening of the 5-mer comprehensive library against **ZW**-conjugated Ki-67.

frkha	nwwtw	efaan	rrhai	fsien
iasln	hrdkh	rrGlh	hrnsh	kaknl
hlGrh	takrk	kyvvk	wynaf	rharl
knwrh	iwkrh	qhaes	ahrr	tpahl
fkrhi	reGlw	kflkn	ihhkw	rhdrn
fkynk	rwhyr	hhkyt	rsqkf	lrslr
ytpaw	vkhrG	nwkrG	ksfwt	lhpkh
rvryh	rykhr	rhyfr	rhwrs	hsfkr
Gdtdw	kyhsh	Gpptl	rfrnh	rrhkd
haskh	ryGyk	arsvr	hvefl	knkka
wykhr	sltts	rksqn	srhfh	hshlr
hknek	arqen	rhhht	hkhff	vkiew
rhhsa	fwhGf	knnhe	hhrqq	kfnhh
rrhqh	kyhnr	hGGhk	dhkhl	rkrid
ytikl	rhhGs	islkd	Ghyft	krvyh
rrywr	hykrf	hhkwt	rkvln	lyyen
whkrw	rnknr	hrhnk	ekkwr	hhisk
fhhhk	rrrnt	vGkrs	hywrr	ywrrh
rrehk	prwhw	kwlwt	dnfhd	wkhqq
Gktrh	frhqr	fhfan	vkyhr	fhvkr
rktaf	iddvl	rGhhs	hrhkd	lsrfi
hGyhr	rrykl	qwknr	rawnt	nrhak
rGfrr	vykrh	dyyff	ehvrl	vkfhh
kwhrh	rhhGh	hkidr	lptfG	kyphr
eqser	wfknr	kkyhf	ryhsr	hahkf
ikfaq	lGdyd	wrktr	kfkeh	kwkfa
rrwhn	frhqr	ihkhl	twdsr	rhqhG
rkfrw	lGkrw	rrlfh	Gpnpk	rhGrh
khytk	ihrrG	lhvGn	hhtki	wGhkr
rpwis	vsitw	ikykq	qhkrp	ykhyh
rhshl	rrnar	sfrGk	rhyrl	rfhqk
rrlnr	ipdyq	peyks	Gskkh	stGkh
ykhna	rwhkd	pkhif	akkwf	kkknk
GkGie				