Electronic Supplementary Material (ESI) for Nanoscale
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SUPPORTING INFORMATION

Table S1: Oligonucleotides used in this study

Name	Sequence
$\mathbf{I^{4}}$	5’-CCCCTAACCCCTAACCCCTAACCCCATATATATCCTAGAACGACAGACAAACAGTGAGTC-3’
$\mathbf{I^{7}}$	5’-CCCCCCCTAACCCCCCCTAACCCCCCCTAACCCCCCCATATATATCCTAGAACGACAGACAAACAGTGAGTC-3’
$\mathbf{I^{C3\text{-}C4}}$	5’-CCCCTAACCCTAACCCCTAACCCATATATATCCTAGAACGACAGACAAACAGTGAGTC-3’
$\mathbf{I^{4'}}$	5’-GACTCACTGTTTGTCTGTCGTTCTAGGATATATATTTTGTTATGTGTTATGTGTTAT-3’
$\mathbf{I^{3}}$	5’-CCCTAACCCTAACCCTAACCC ATATATATCCTAGAACGACAGACAAACAGTGAGTCCGCATTGTTACAT-3’
$\mathbf{I^{3'}}$	5’-ATGTAACAATGCGGACTCACTGTTTGTCTGTCGTTCTAGGATATATAT TTGTTAGTGTTAGTGTTAT-3’
$\mathbf{I\text{-}^{C\text{-}myc}}$	5’-CCCCACCCTCCCCACCCTCCCCATATATATCCTAGAACGACAGACAAACAGTGAGTC-3’
$\mathbf{I\text{-}^{C\text{-}myc'}}$	5’-GACTCACTGTTTGTCTGTCGTTCTAGGATATATATTTTTGTGAGTGTGTGTAG-3’
$\mathbf{I^{4}_{A488}}$	5’-Alexa 488—CCCCTAACCCCTAACCCCTAACCCCATATATATCCTAGAACGACAGACAAACAGTGAGTC-3’
$\mathbf{I^{3}_{A488}}$	5’-Alexa 488—CCCTAACCCTAACCCTAACCCATATATATCCTAGAACGACAGACAAACAGTGAGTC-3’
$\mathbf{I^{7}_{A488}}$	5’-Alexa 488—CCCCCCCTAACCCCCCCTAACCCCCCCTAACCCCCCCATATATATCCTAGAACGACAGACAAACAGTGAGTC-3’

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I^4_{A647}	5'-GACTCACTGTTTGTCTGTCGTTCTAGGA**T**ATATAT<u>TTT</u>GTTATGTGTTATGTGTTAT-3' (T --Alexa 647)
ssDNA	5'-Biotin--AAAAGACTCAC TGTTTGTCTGTCGTTCTAGGATATATAT-3'
ssDNA′	5'-ATATATATCCTAGAACGACAGACAAACAGTGAGTC-3'
Region 1	5'-ATATATATCCTAG-3'
Region 2	5'-CGACAGACAAACA-3'
Region M	5'-CCTAGAACGACAG-3'
pHEN seq	5'-CTA TGC GGC CCC ATT CA-3'

Table S1: Sequences used and associated nomenclature of samples in the present study. I switch devices incorporating a pH responsive segment Cn-Cn-Cn-Cn are referred to as I^n when they carry no fluorescent labels. Thus, $\mathbf{I^3}$ is formed from a 1: 1 mixture of I^3 and $I^{3'}$, $\mathbf{I^4}$: I^4 and $I^{4'}$, $\mathbf{I^7}$: I^7 and $I^{4'}$, $\mathbf{I^{3\text{-}4}}$: $I^{3\text{-}4}$ and $I^{4'}$, $\mathbf{I^{c\text{-}myc}}$: $I^{c\text{-}myc}$ and $I^{c\text{-}myc'}$. Fluorescently labeled I-switch assemblies indicate the respective fluorophore in the subscript, where e.g., $\mathbf{I^3_{A488/A647}}$ is formed from I^3_{A488} and $I^{3'}_{A647}$, $\mathbf{I^4_{A488/A647}}$: I^4_{A488} and $I^{4'}_{A647}$, $\mathbf{I^4_{A488}}$: I^4_{A488} and $I^{4'}$. The 35 bp dsDNA epitope is formed from ssDNA and ssDNA′, $\mathbf{R_1}$: ssDNA and Region 1, $\mathbf{R_2}$: ssDNA and Region 2, $\mathbf{R_M}$: ssDNA and Region M.

Design of DNA pH sensors

Please note that, a stretch of thymines (underlined in Table S1) are introduced to insulate the pH sensing domain (purple, main text Fig. 1) and the handle domain (gray, Fig. 1).

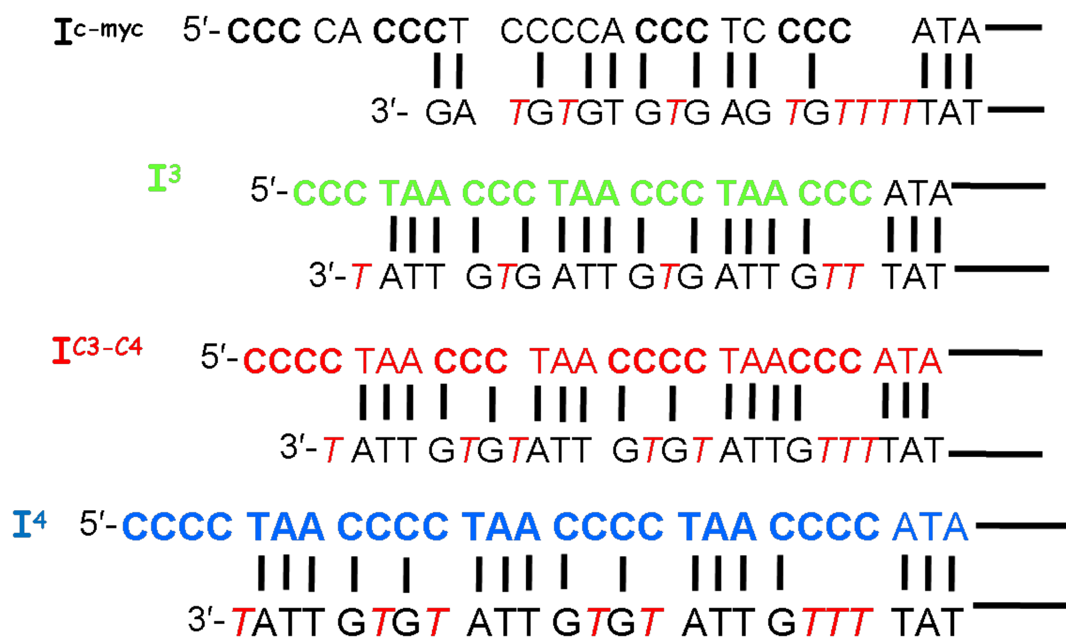

Fig. S1. pH sensing domain of four representative DNA pH sensors showing paired regions (black lines) and engineered mismatches.

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I-switch conformational change probed by FRET

In order to investigate I-switch folding by FRET, the two strands were labeled with two fluorophores which act as a FRET pair. I^4 was labeled with Alexa 488 at $5'$ end whereas $I^{4'}$ was labeled with Alexa 647 internally. When fluorescence spectra of samples composed of donor only (I^4_{A488}), acceptor only (I^4_{A647}) and dual-labeled ($I^4_{A488/A647}$) I-switch at pH 5 were acquired, only the dual-labeled sample showed high FRET confirming i-motif folding (Fig. S2).

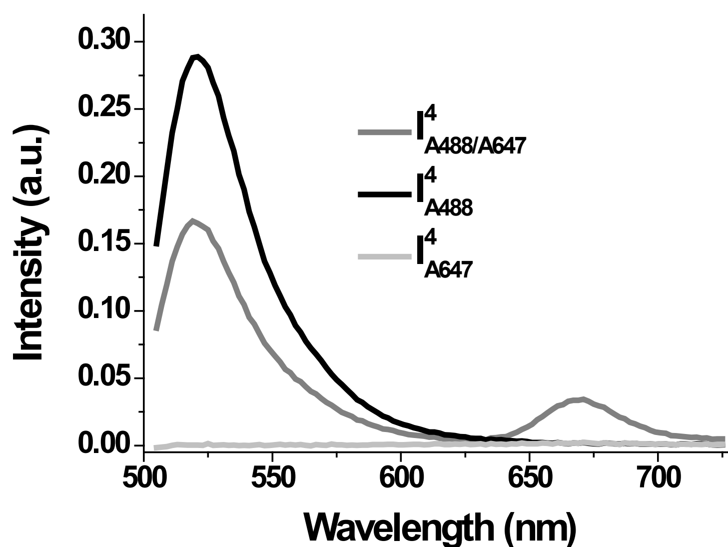

Fig. S2. Steady state fluorescence measurements on I-switch. Fluorescence spectra of differently labeled I-switches demonstrate i-motif folding. 50 nM of labeled switch was diluted in 20 mM phosphate buffer of pH 5.0 containing 100 mM KCl, incubated for 30 min before acquiring spectra. Samples were excited at 488 nm and fluorescence spectra was recorded from 505 nm to 725 nm.

Phage display technique to generate recombinant antibodies against DNA duplex

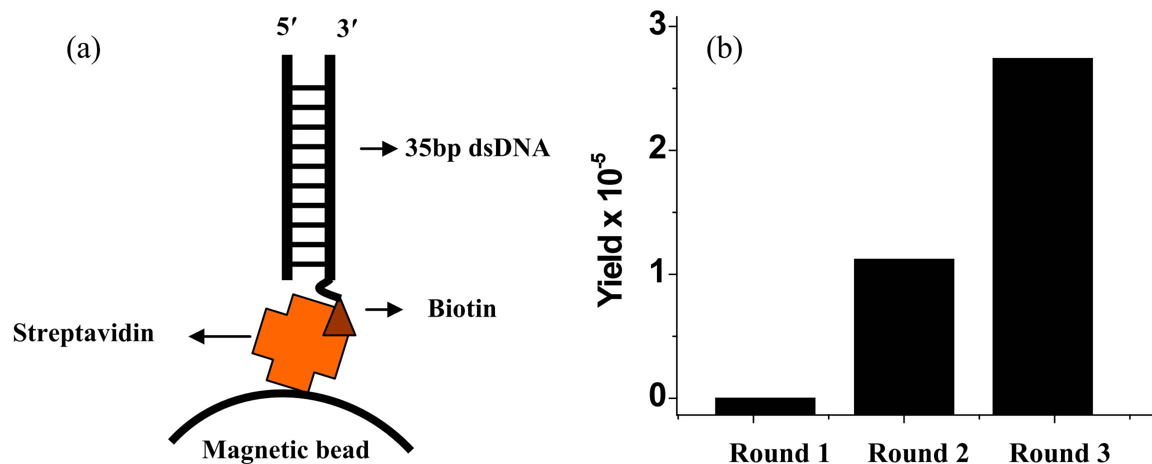

Fig. S3: Screening of dsDNA binding scFvs. (a) Schematic of dsDNA epitope used for screening. (b) Yield of phage after each round of selection was shown by counting colonies present in different dilutions of eluted phage particle after triethyl amine treatment. Different dilutions of eluted phage was used to infect TG-1 bacteria and plated in 2×TY plates containing 100 µg/mL ampicillin and 1% glucose. After 16 h growth number of TG-1 colonies present in the plate was counted and fed onto the algorithm described earlier.[3]

Selection of sequence-specific scFvs

Sequence specific scFvs were identified by dividing the 35bp dsDNA into three regions. One region was composed of the 13 base pairs of the antigen from 3′ end (known as Region 1 (R_1), Fig. S4a, red), the second region corresponded to base pairs 16 to 28 from 3′ end (Region 2 (R_2), Fig. S4a green) and third region was an overlap of regions 1 and 2 (Region M (R_M), Fig. S4a, blue). These three duplexes were annealed and then immobilized on three different 96-well plates. scFv supernatant of the same clones were added to the wells and incubated as described in earlier. It was observed that clone C1 was specific for R_1 (Fig. S4b(i)), in that they did not bind R_2 (Fig. S4b(ii)) or the R_M (Fig. S4b(iii)). Thus, it is fair to conclude that the scFvs corresponding to these clones recognize only the first 8 base pairs of the 35bp dsDNA i.e., the sequence $d(AT)_4$. Careful analysis revealed that 14 clones were specific for R_1, 3 clones were specific for R_2. We also found few clones that bind all three regions (Fig. S4b(iv)), showing no sequence specificity, which we did not include for further analysis.

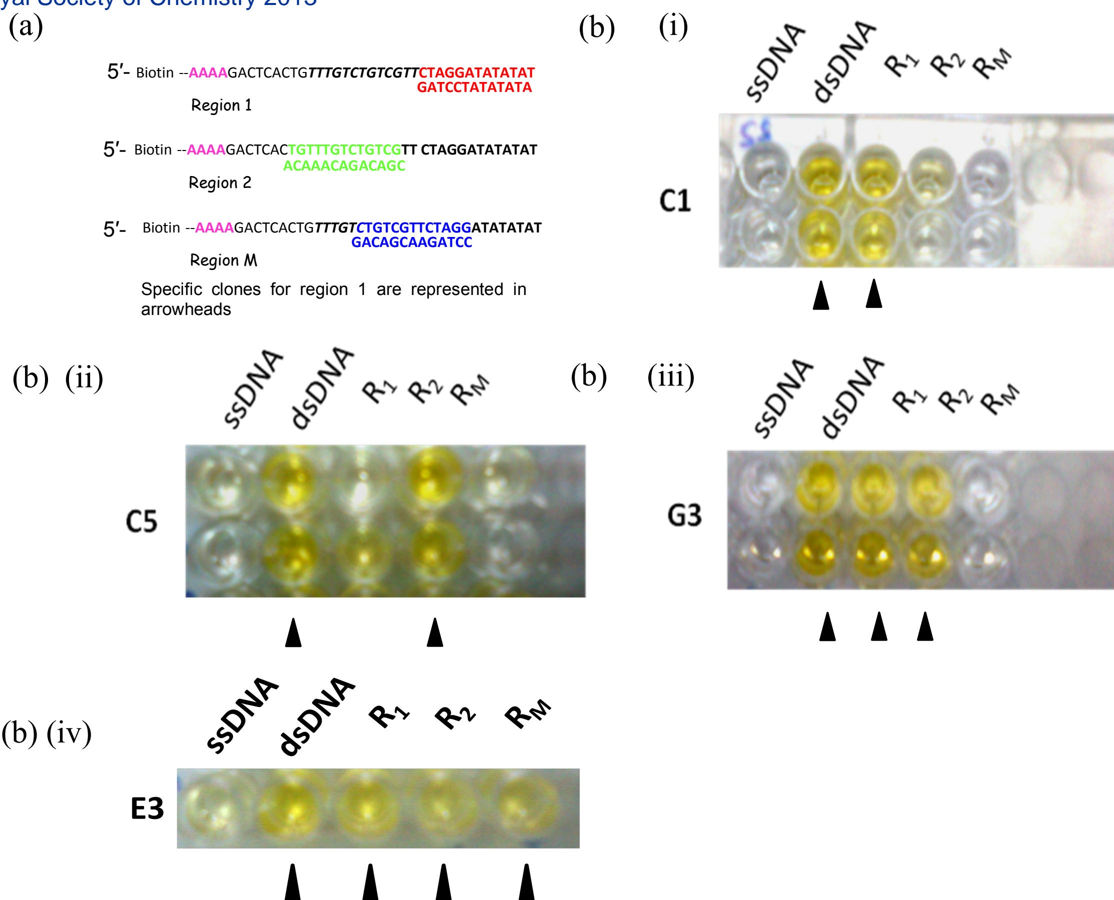

Fig. S4. Screening of DNA sequence-specific scFvs. (a) Schematic of DNA strands used in sequence specific ELISA screens. R_1(red font), R_2 (green font) and R_M (blue font). (b(i-iii)) ELISA results obtained after incubating protein supernatant of indicated clones with epitopes shown in (a): (i) Region R_1 specific clone, (ii) R_2 specific clone, (iii) R_1 and R2 specific clone (iv) Not specific to a given sequence. Arrows are indicative of positive clones specific for respective regions.

scFv expression and purification

scFv C1 was chosen for further studies. This scFv contains the bacterial pel B signal sequence at the N-terminus that secretes scFv into the periplasm where the pelB signal sequence is removed and scFv secreted into the medium. When protein was expressed in either 2×TY or M9 minimal media and subjected to a purification involving Ni-NTA or HisPur Cobalt Resin it showed a single band near 27 kDa. When all the fractions were analyzed by the SDS-PAGE, maximum abundance was seen in fractions 2–4 (Fig. S5). These fractions were pooled and the concentration of scFv was measured by Bradford assay. A typical preparation yielded 0.5-2 mg/300 mL protein

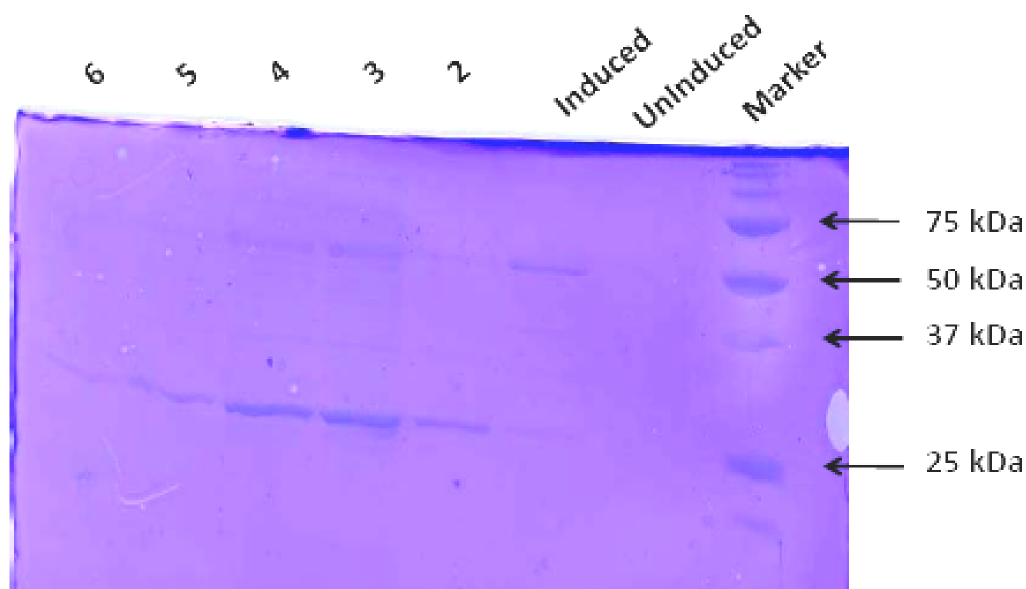

Fig. S5. scFv production and purification. Clone C1 was expressed in M9 media supplemented with 0.1% casamino acids and 0.2% glycerol. Marker: Precision plus protein marker (Bio-Rad); Uninduced: supernatant of uninduced culture; Induced: supernatant of culture induced by 1 mM IPTG; 2–6: fractions 2–6 collected post elution with 500 mM imidazole.

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Binding studies with scFv and 35bp dsDNA epitope

Affinity of scFv C1 with the 35 base pair DNA duplex (main text Fig. 3a) was studied by ELISA in three formats that (i) used varying dsDNA concentration, (ii) used varying scFv concentration. When concentration of immobilized dsDNA was varied while probing with a fixed concentration of scFv (3 μM, 100 μL), a plot of UV absorbance at 450 nm (OD_{450}) as a function of of DNA yielded a sigmoidal curve. Normalization to the fraction of bound scFv yielded an IC_{50} value of 31±5.8 nM indicating a high affinity complex between scFv and DNA (Fig. S6a). When immobilized DNA amount (25 pmoles) was kept constant and scFv concentration was varied and OD_{450} was plotted as a function of scFv concentration. This yielded a sigmoidal curve which was fitted regression analysis to a single site binding isotherm providing a K_D ~212 nM ((Fig. S6b).

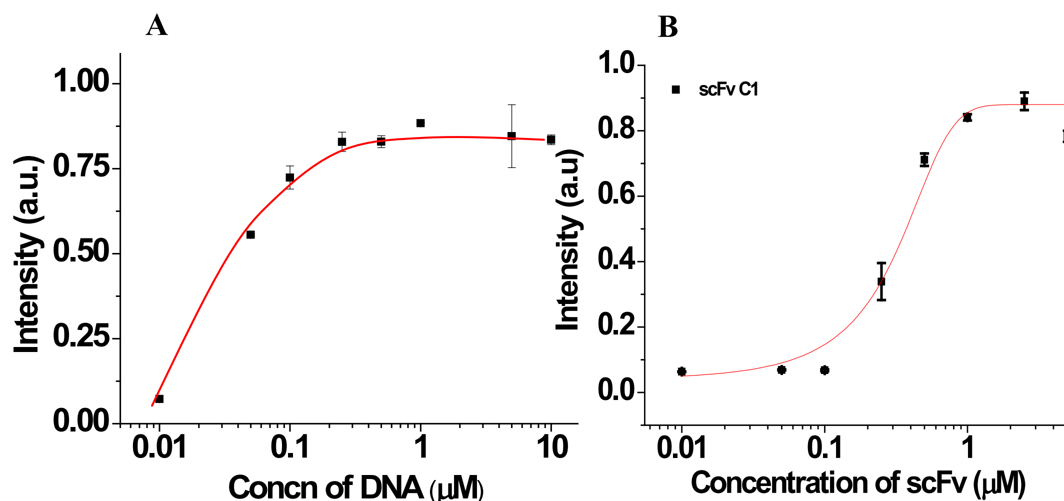

Fig. S6. Determination of binding constant using scFv C1 with 35bp dsDNA. (a) Biotinylated 35bp dsDNA was immobilized at different concentrations with a constant scFv amount (300 pmoles). (b) Various concentrations of scFv were added to the wells containing dsDNA (25 pmoles) immobilized at the same concentration. After dsDNA-scFv incubation, binding was measured using ELISA and absorbance at 450 nm was normalized with respect to the highest observed intensity and plotted as a function of concentration. The lines are intended only for visual clarity.

Clamping of I-switch internalized HeLa cells at different pH

scFv expressing HeLa cells were labeled with 500 nM $I^4_{A488/A647}$ for 15-30 minutes at 37°C in complete media. Cells were washed and briefly fixed for 2 min in ice using 2% paraformaldehyde. Cells were pH clamped using clamping buffer containing 25-40 μM nigericin. Cells were imaged in a widefield microscope after exciting at 488 nm and imaging at 520 nm (D) and 669 nm (A). D and A images were aligned and a binary image was created by dividing D images by A images. This binary D/A image was further pseudocolored in ImageJ to provide a spatial pH map of cells clamped at indicated pHs. As expected, due to high FRET, at low pH cells showed uniformly lower D/A which concomitantly increased to higher D/A value as value of clamping buffer pH increased.

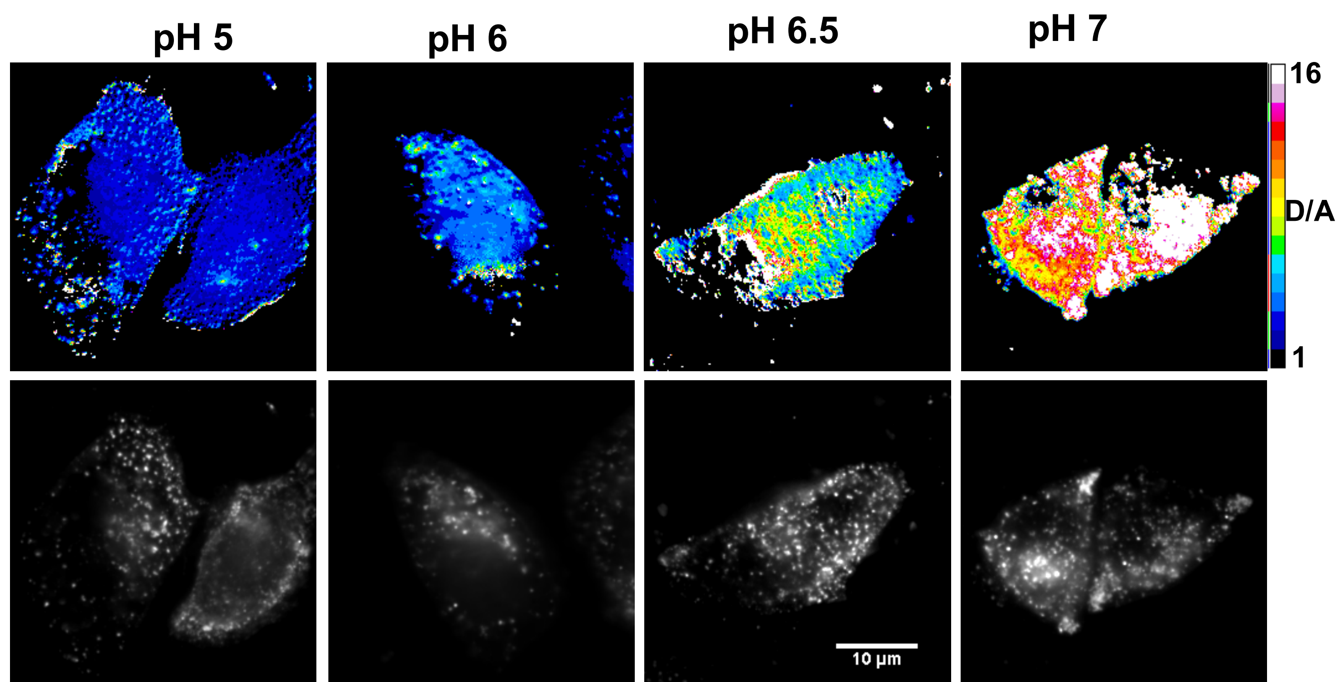

Fig. S7. *In cellulo* performance of $I^4_{A488/A647}$. Representative pseudocolour D/A map of HeLa cells clamped at indicated pH values is shown along with their respective fluorescence images (in 520 channel) shown in grayscale. scFv-furin expressing HeLa cells were labeled with $I^4_{A488/A647}$, and briefly fixed in ice with 2% PFA for 2 min followed by addition of clamping buffer of different pH. Cells were then imaged after 40 min of incubation in clamping buffer of the indicated pH. Scale Bar: 10 μm.

I-switch is internalized along the furin endocytic pathway.

Furin is predominantly present in the TGN of which a small population shuttles between the plasma membrane and TGN via the sorting endosome and late endosome.[4] To see whether the I-switch marks the furin retrograde pathway, colocalization experiments with molecular markers of sorting endosome and late endosome were performed. First, the sorting endosomes in the scFv-furin expressing HeLa cells were labeled with a 10 min pulse containing a cocktail of Alexa 568 labeled transferrin (Tfn_{568}) and $I^4_{A488/A647}$ and imaged. It was observed that Tfn_{568} and $I^4_{A488/A647}$ showed significant colocalization indicating that after endocytosis, most of the I-switch is resident in sorting endosomes (Fig. S8a,c).

In order to check the identity of the compartments at the 2 h timepoint, scFv expressing HeLa cells were labeled with a mixture of $I^4_{A488/A647}$ and TMR-Dextran. scFv-furin expressing HeLa cells were labeled with $I^4_{A488/A647}$ and TMR dextran for 1.5 h, washed, chased for 3h and imaged. At this time point $I^4_{A488/A647}$ and TMR dextran showed significant colocalization indicating that $I^4_{A488/A647}$ had trafficked from sorting endosome into the late endosome, characteristic of furin mediated retrograde trafficking (Fig. S8b,d).

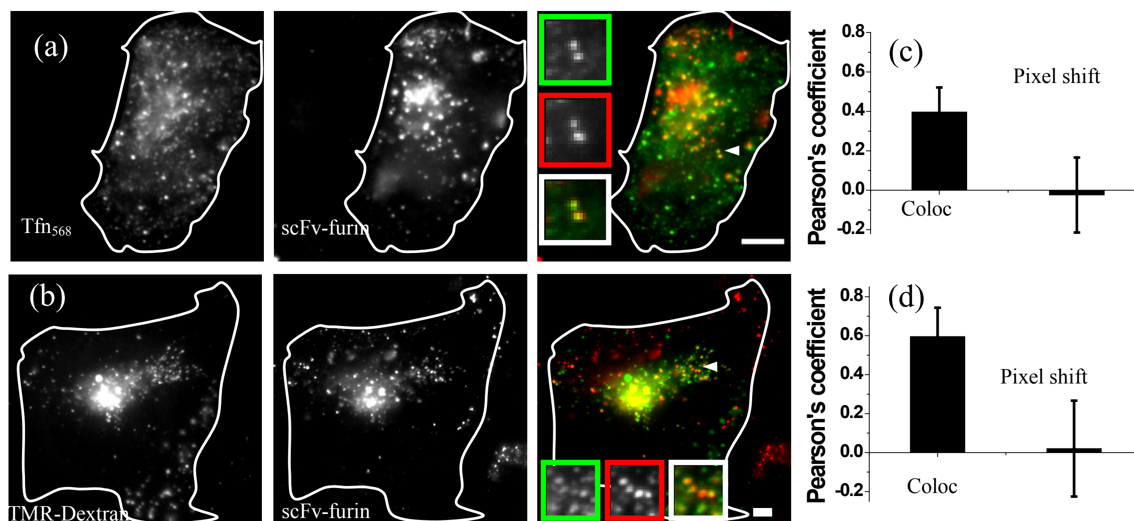

Fig. S8. *In cellulo* trafficking of I-switch. (a) scFv-furin expressing HeLa cells pulsed with $I^4_{A488/A647}$ and Transferrin-A568 (Tfn_{568}) for 10 min at 37°C, washed and imaged immediately. (b) HeLa cells were pulsed with TMR-Dextran and $I^4_{A488/A647}$ at 37°C for 1.5 h chased for 3 h and then imaged. (c-d)

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Quantification of colocalization between $I^{4}_{A488/A647}$ and endosomal markers used in (A-B). Experiments were performed in duplicate. Error Bar: Mean of >10 cells ± SD. Scale bar 5 μm.