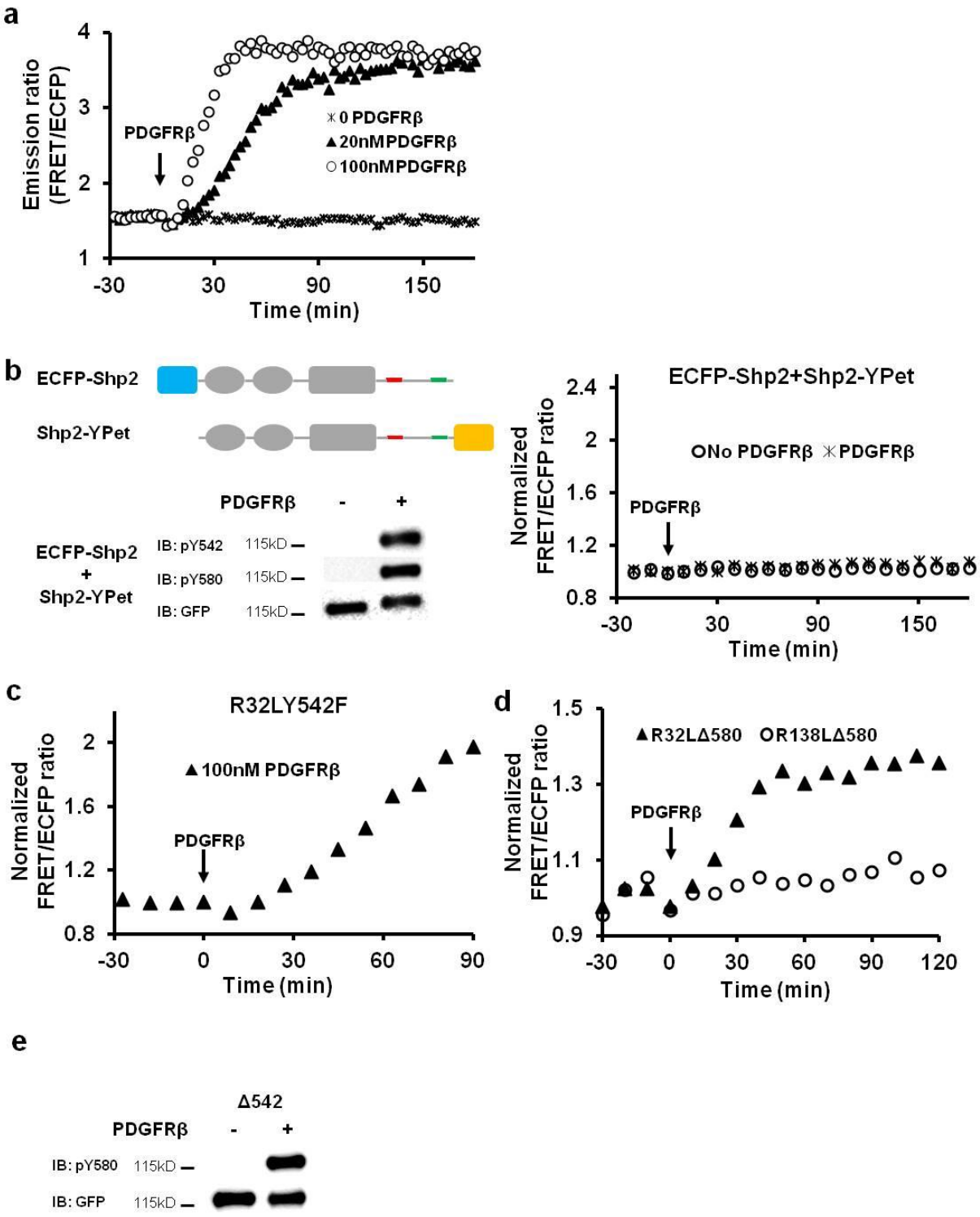
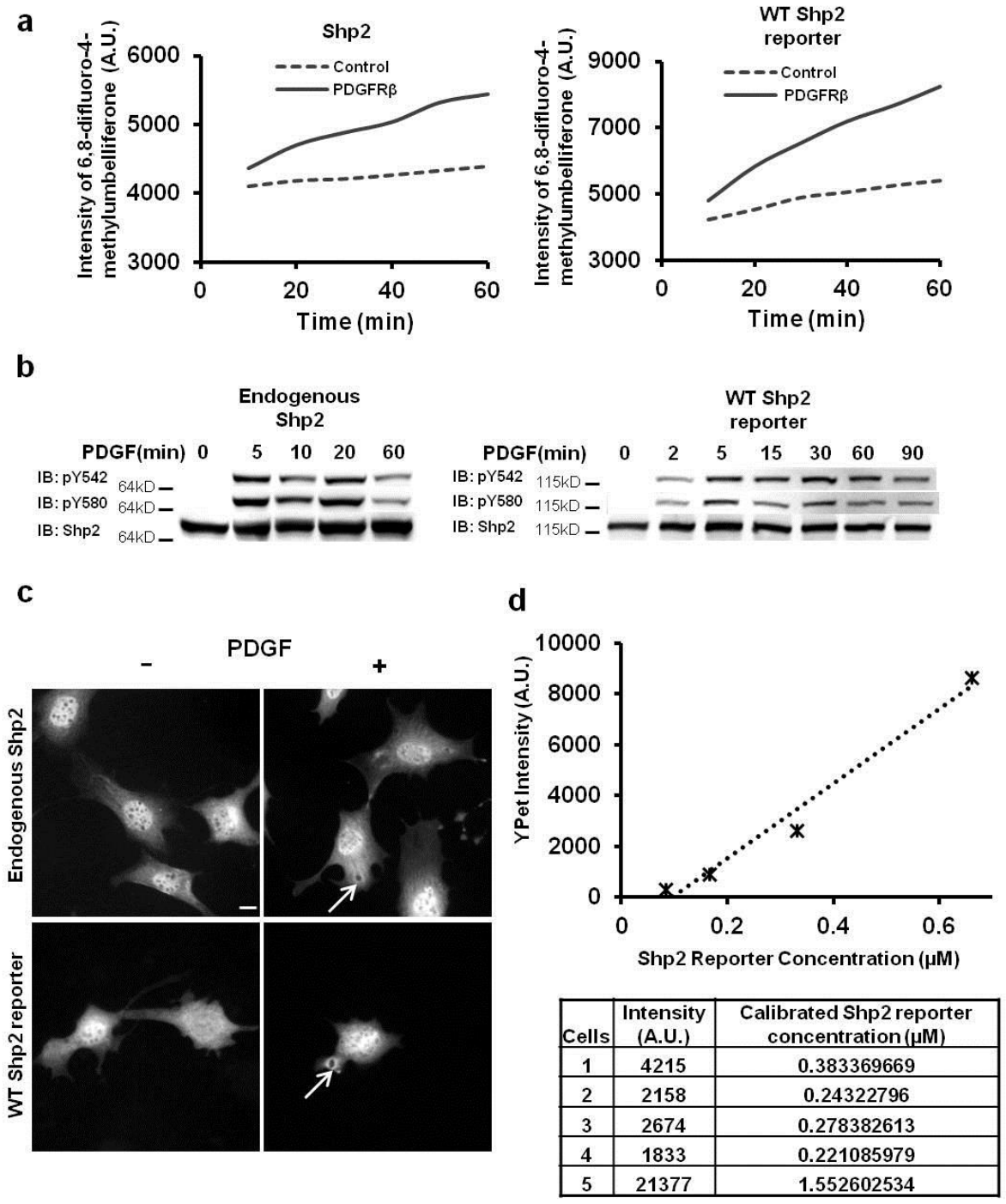
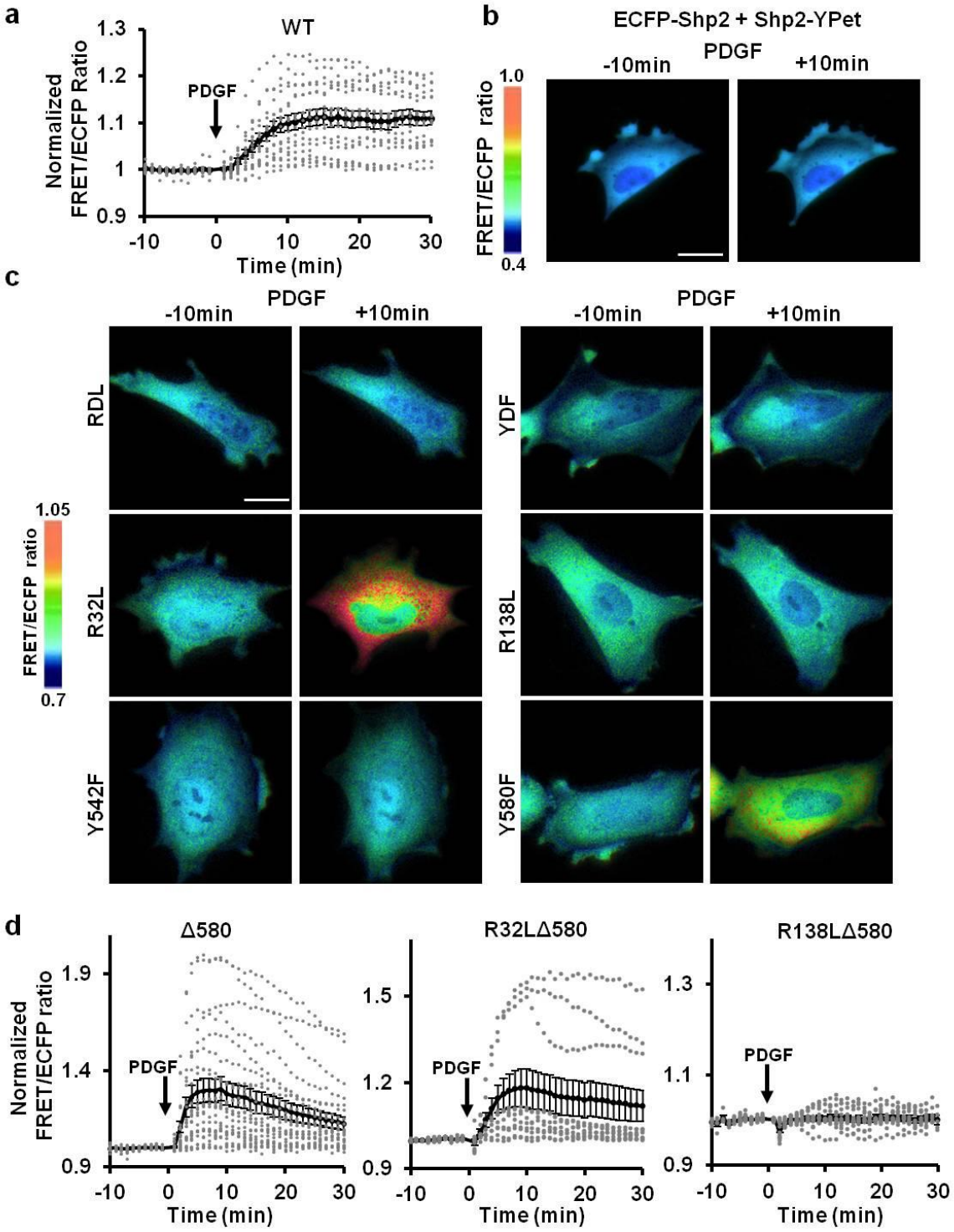


Supplementary Figure S1: Characterization of Shp2 reporters *in vitro*. (a) The time courses of FRET/ECFP ratio of WT reporter incubated with different concentrations of PDGFRβ kinase. (**b**) Left: upper panels show the domain structure of Shp2 fused with donor or acceptor separately (ECFP-Shp2 and Shp2-YPet); lower panels show the tyrosine phosphorylation of these fusion proteins before and after 180 min PDGFRβ incubation. Right: the time courses of normalized FRET/ECFP ratio of mixed solution containing both ECFP-Shp2 and Shp2-YPet, with or without the incubation of PDGFRβ kinase. The time courses of normalized FRET/ECFP ratio of (**c**) R32LY542F, (**d**) R32LΔ580 or R138LΔ580 reporter before and after PDGFRβ kinase incubation. (**e**) The phosphorylation of Δ542 reporter before and after 180 min PDGFRβ incubation.

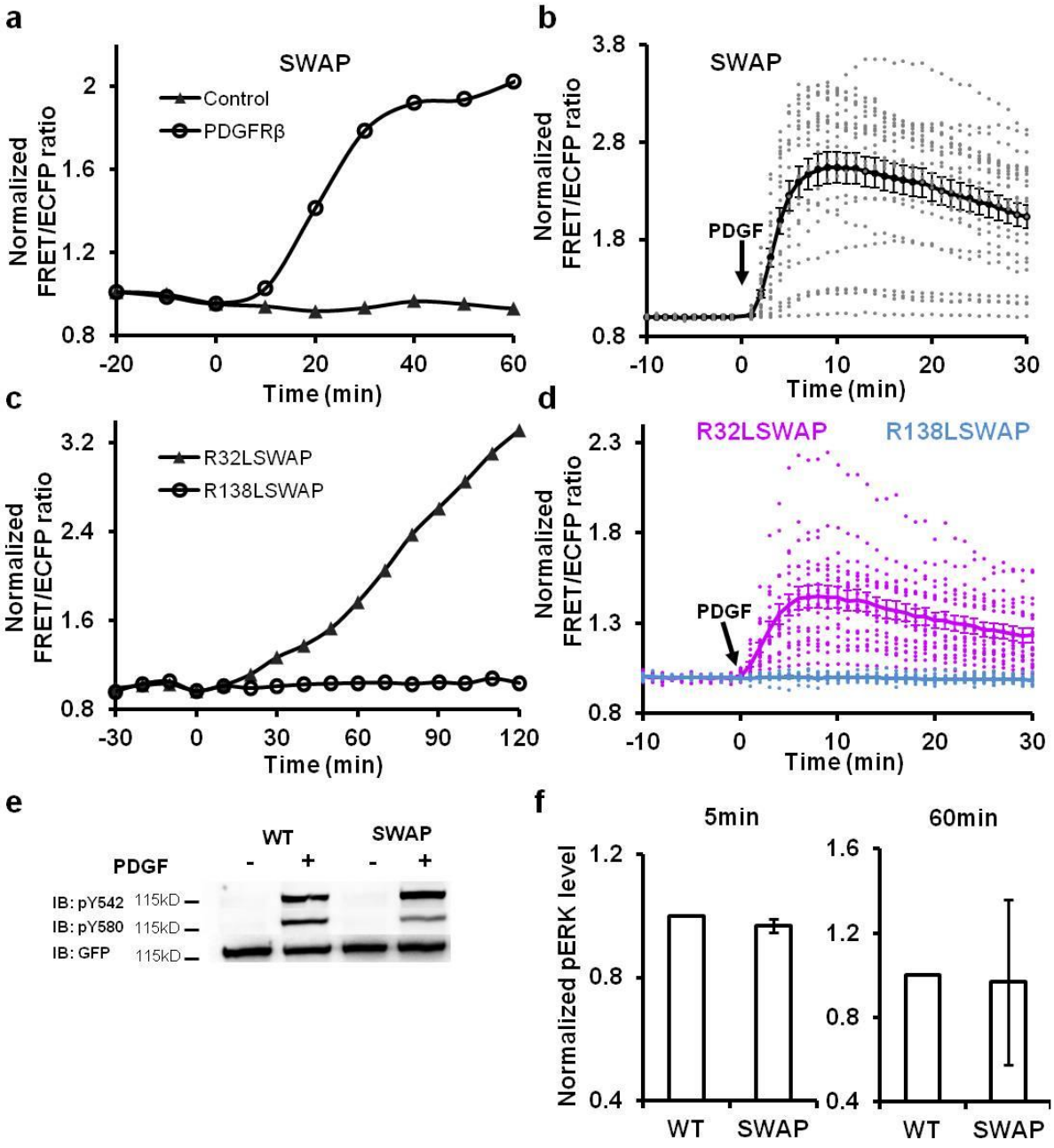


Cells	Intensity (A.U.)	Calibrated Shp2 reporter concentration (μM)
1	4215	0.383369669
2	2158	0.24322796
3	2674	0.278382613
4	1833	0.221085979
5	21377	1.552602534

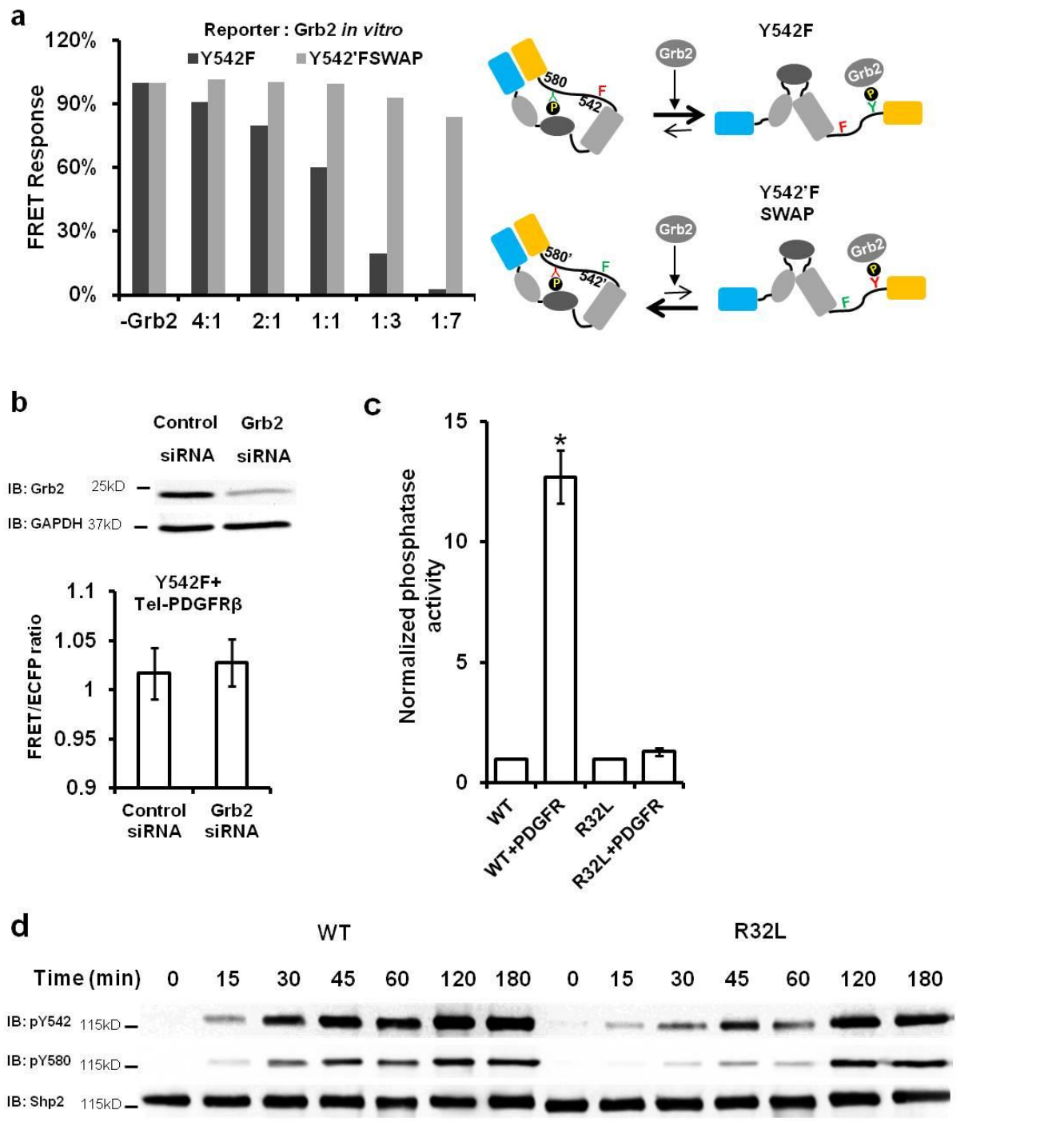
Supplementary Figure S2: The comparison of Shp2 reporter with endogenous Shp2. (**a**) The time courses of fluorescence intensity of 6,8-difluoro-4-methylumbelliferone (product from DiFMUP) to represent the phosphatase activities of purified His-tagged full-length Shp2 (left) and WT Shp2 reporter (right) with ("PDGFRβ") or without ("control") PDGFRβ incubation. (**b**) The top two gel panels show the tyrosine phosphorylation of Y542 and Y580 of the endogenous Shp2 (left) or WT Shp2 reporter (right) in MEFs before and after PDGF stimulation. The bottom panel shows the equal amount of proteins loaded under different conditions. (**c**) Immunostaining images of endogenous Shp2 and WT Shp2 reporter in MEFs before and after 5 min PDGF stimulation. White arrows point to membrane ruffles. Scale bar = 10μm. (**d**) The estimation of cellular concentration of Shp2 reporter in MEFs. Top graph shows YFP intensity of the purified Shp2 reporter with known concentrations. The bottom table shows calibrated cellular concentrations of the Shp2 reporters in five different cells.



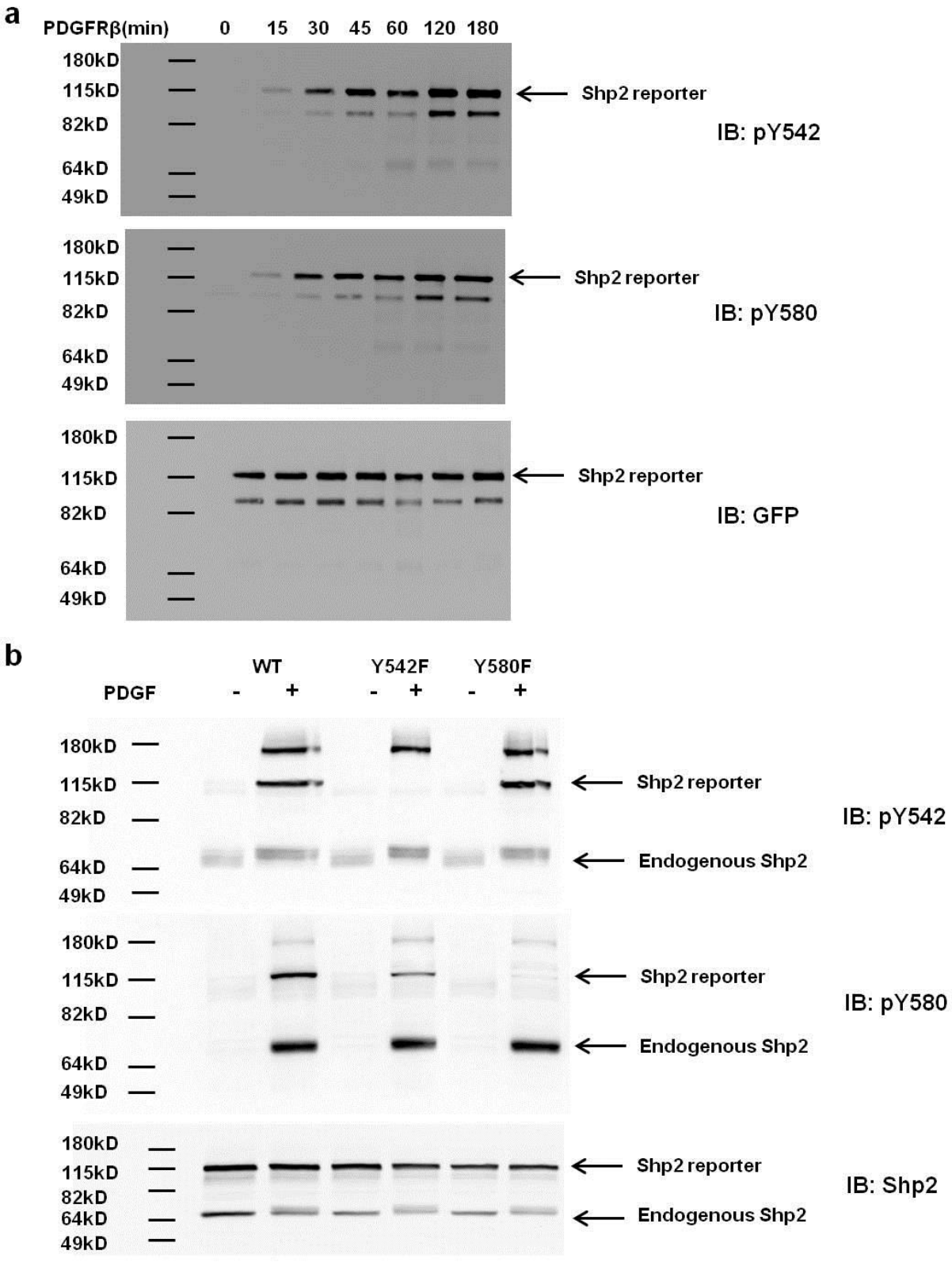
Supplementary Figure S3: Characterization of Shp2 reporters in MEFs. (**a**) The time courses of the normalized FRET/ECFP ratio from each individual MEF cell expressing WT reporter (grey dots), with the average curve and its standard errors shown in solid black. (**b**) The FRET/ECFP emission ratio images of MEF cells transfected with both ECFP-Shp2 and Shp2-YPet reporters before and after PDGF stimulation. Scale bar =10 μm. (**c**) The FRET/ECFP emission ratio images of MEF cells transfected with different mutant reporters as indicated before and after PDGF stimulation. Scale bar = 10 μm. (**d**) The time courses of the normalized FRET/ECFP ratio of Δ580, R32LΔ580, and R138LΔ580 reporters in MEFs before and after PDGF stimulation (grey dots), with the average curve and its standard errors shown in solid black. Error bars indicate S.E.M.



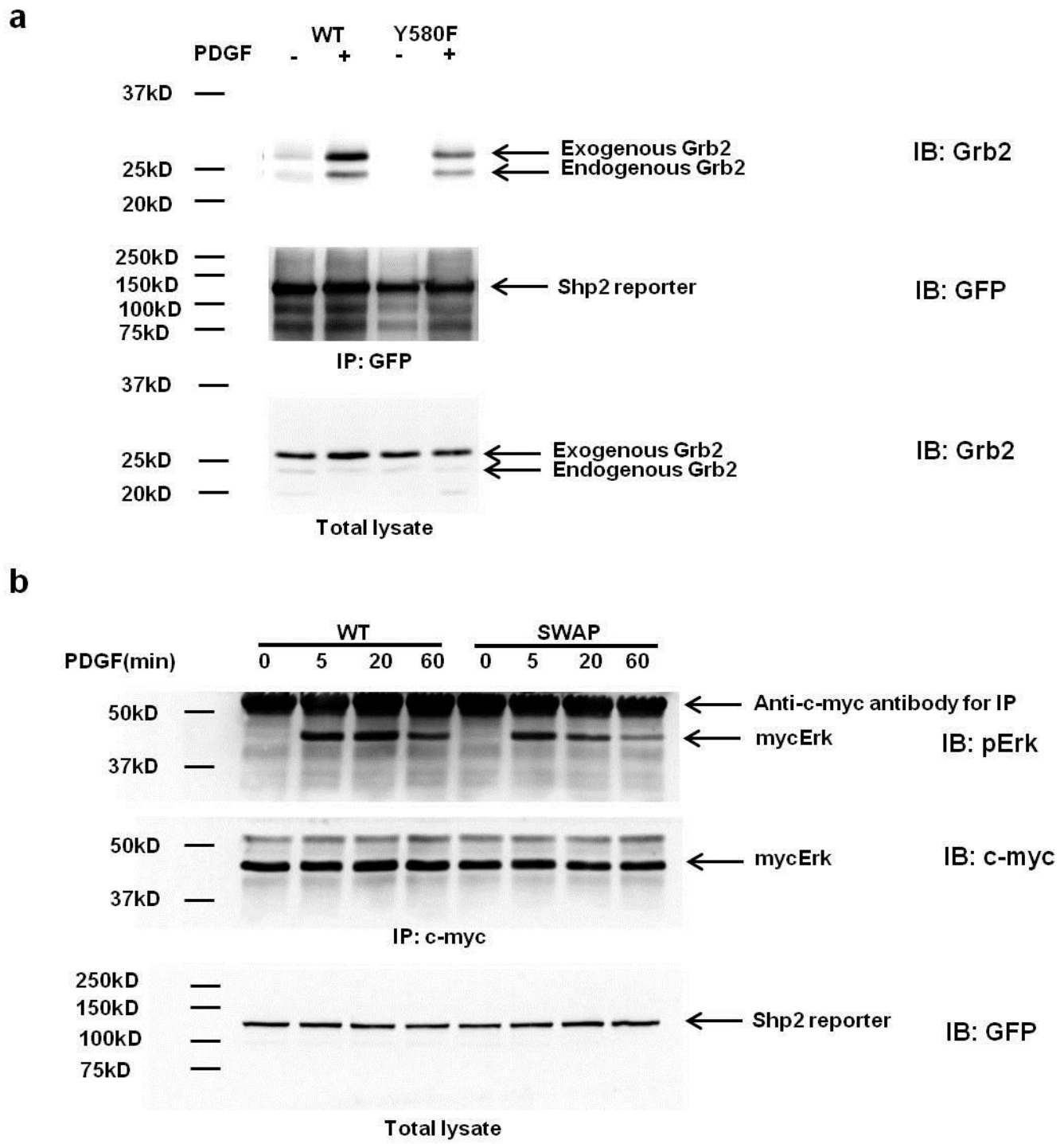
Supplementary Figure S4: Characterization of SWAP reporters. (**a**) The time courses of normalized FRET/ECFP ratio of SWAP reporter *in vitro* with ("PDGFRβ") or without ("Control") PDGFRβ incubation. (**b**) The time courses of the normalized FRET/ECFP ratio from each individual MEF cell expressing the SWAP reporter (grey dots), with the average curve and its standard error shown in solid black. (**c**) The time courses of normalized FRET/ECFP ratio of R32LSWAP or R138LSWAP reporter *in vitro* before and after PDGFRβ incubation. (**d**) The time courses of the normalized FRET/ECFP ratio from each individual MEF cell expressing the R32LSWAP (purple) or R138LSWAP (blue) reporter before and after PDGF stimulation (dots), with the average curves and standard errors shown in solid lines. (**e**) The top two gel panels show the tyrosine phosphorylation of Y542 (or Y580') and Y580 (or Y542') of the WT or SWAP reporter before and after 5 min PDGF stimulation in MEFs. The bottom panel shows the equal amount of reporter proteins loaded under different conditions. (**f**) The graphs show the normalized pERK level of MEFs expressing either WT or SWAP reporter 5 min (left) or 60 min (right) after PDGF stimulation. There is no statistical difference observed among different groups compared.



Supplementary Figure S5: The effect of Grb2 on Shp2 reporters. The FRET response of Y542F and Y542'FSWAP reporters to PDGFRβ kinase *in vitro* with or without the presence of different Grb2 amounts. The right panel represents the schematic drawings of different conformational responses of the Y542F and Y542'FSWAP reporters upon the Grb2 addition. (**b**) Upper panel, siRNA specific for Grb2 decreases the expression level of endogenous Grb2 compared to control siRNA. Lower panel, Grb2 knockdown by siRNA doesn't decrease the FRET signal of Y542F reporter. P=0.77. (**c**) The phosphatase activity of Shp2 reporters in response of. PDGFR incubation leads to an increase of PTPase activity as high as 12.7 fold in WT reporter while R32L mutation in N-SH2 domain abolished this increase *in vitro*. (**d**) WT and R32L reporter can both be phosphorylated at Y542 and Y580 *in vitro*.



Supplementary Figure S6: Representative western blots. The uncropped whole blots of (**a**) Figure 1b and (**b**) Figure 3c. The full length Shp2 reporter is around 120kD.. Endogenous Shp2 has a molecular weight around 68kD.



Supplementary Figure S7: Representative Western blots. The uncropped whole blots of (**a**) Figure 5e and (**b**) Figure 6e. The exogenous Grb2 has a higher molecular weight than endogenous Grb2 due to the His-tag and surrounding sequences in the expression vector. The thick band above 50 kD in (**b**) is the heavy chain of antibody used for IP.

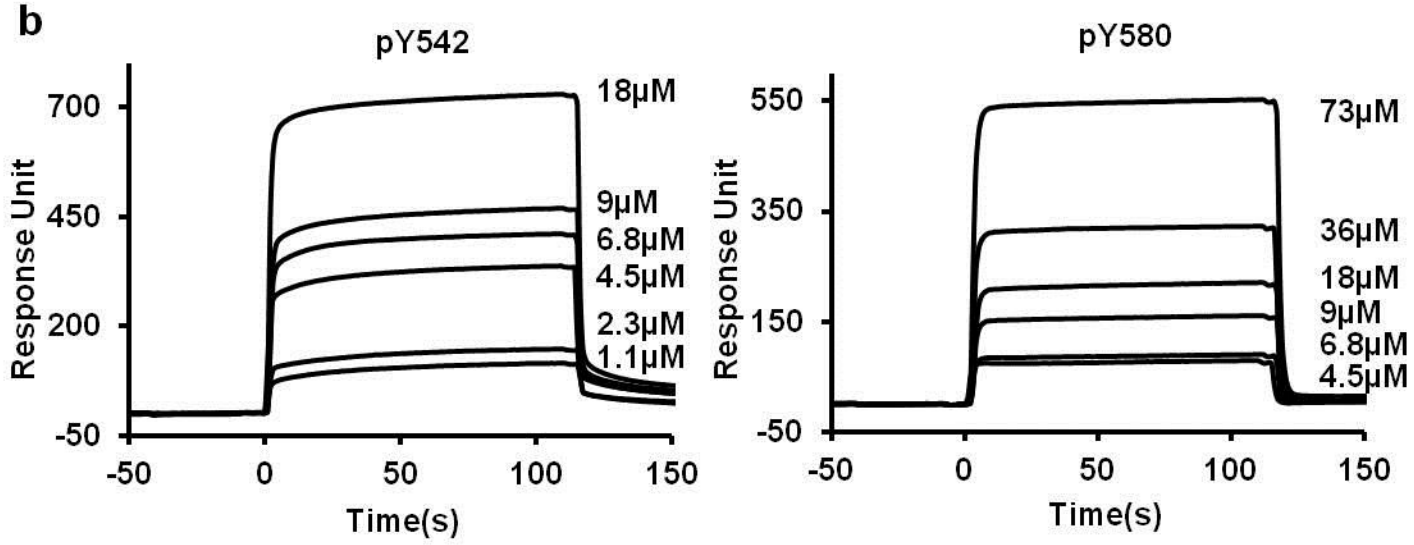
a

Association rates Kon of peptides toward C-SH2 domain

Peptide name	Peptide sequence[a]	Kon ($x10^3$ $M^{-1}S^{-1}$)
pY542	KGHEpYTNIKYS	3.780 ± 0.684
pY580	SARVpYENVGLM	0.891 ± 0.122

a All peptides contained a biotin-ASASA at their N termini

b



Supplementary Figure S8: Representative sensorgrams. (**a**) The association rates of peptides toward C-SH2 domain. (**b**) The original sensorgrams showing the binding of C-SH2 domain to either pY542 or pY580 peptide.