Supplemental Materials

Molecular Biology of the Cell

Wills et al.

Supplemental Figures

Fig. S1. Anti-pEGFR Y1068 antibody detects only the phosphorylated form of the EGFR protein. The specificity and cross reactivity of an antibody raised against EGFR phospho-tyrosine Y1068 was evaluated by immunoblot and immunofluorescence. (A) Lysate collected from unstimulated (T=0) and 50 ng/ml EGF-stimulated (T=10) COS-1 cells was immunoblotted for EGFR pTyr1068. (B) When ShcD-GFP transfected cells were exposed to EGF and visualized by confocal microscopy, robust pY1068 fluorescence was observed. Conversely, introduction of ShcD and a constitutively active kinase binding partner into HEK 293T cells, which do not express endogenous EGFR, failed to elicit a pEGFR signal.

Fig. S2. ShcD-induced EGFR phosphorylation is comparable in COS-1 and HEK-293 cells. The capacity of COS-1 to activate endogenous EGFR in the presence of ShcD was compared to the response of HEK 293 cells overexpressing both EGFR and ShcD. Total protein levels were equalized prior to loading.

Fig. S3. MS/MS spectra for all EGFR phospho-tyrosine peptides. The MS/MS database search and software/method used to evaluate site assignment were described in experimental section. The peptide sequence, the precursor m/z and charge observed, and the score/E-value for the peptide were all listed in supplementary Table 2.

Supplemental Tables

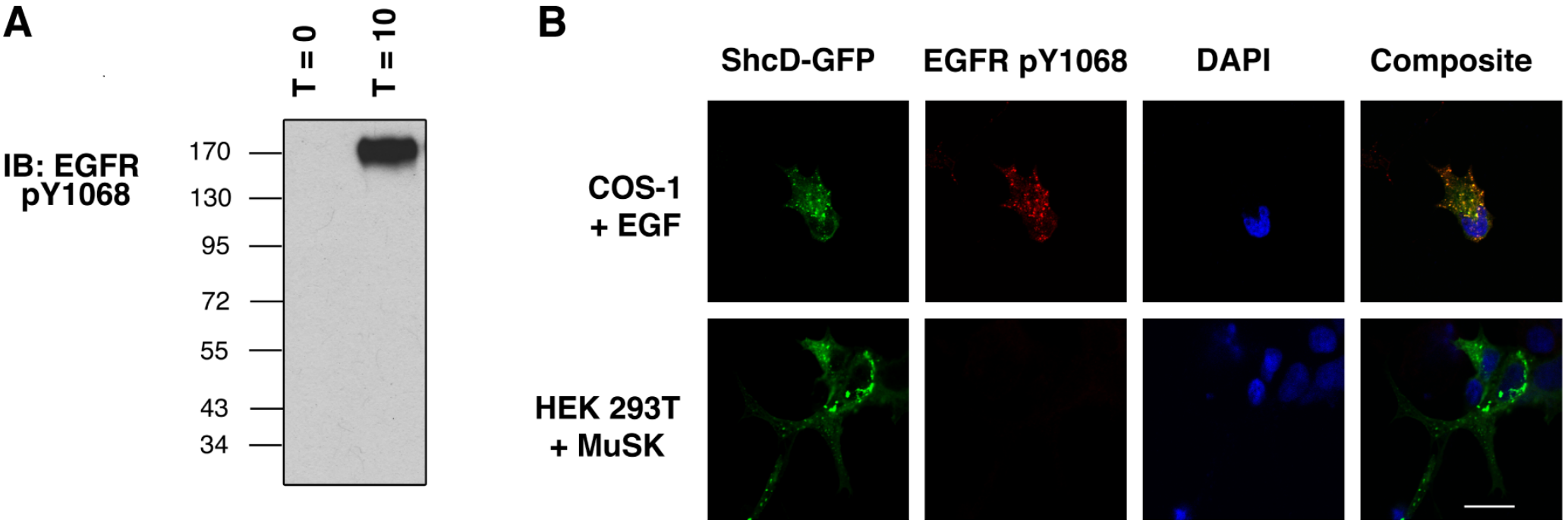
Table S1. Co-localization of ShcD (green) and pEGFR (red) signals expressed as Pearson's correlation coefficient, with comparison to van Steensel randomized signal.

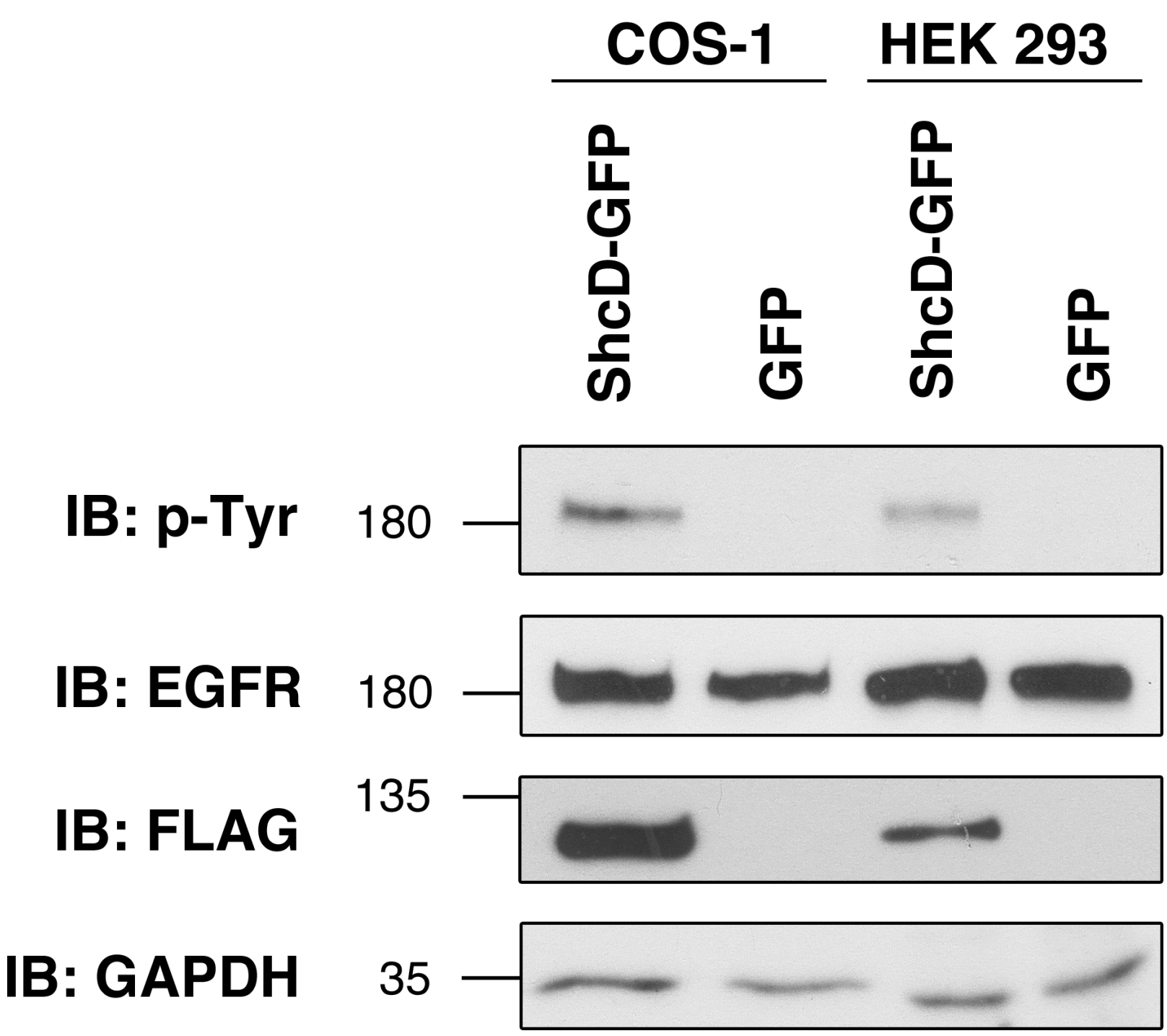
			Van Steensel x-Translation	
Transfection	**Time (min)**	**Pearson's R**	**R(rand) mean +/- SD**	**R(Obs) > R(rand) %**
ShcD-GFP	T = 0	0.790	0.488 +/- 0.162	95.10%
ShcD-GFP	T = 60	0.820	0.552 +/- 0.138	97.60%
ShcD-GFP	T = 120	0.855	0.551 +/- 0.113	97.60%
GFP	T = 10	0.147	0.139 +/- 0.009	80.50%

Table S2. MS information for EGFR phosphopeptides.

EGFR Peptide Sequence*	**p-Tyr sites**	**SEQUEST XCorr score**	**X!Tandem - log(e)**	**Observed m/z**	**Charge (e)**
(R)YSSDPTGALTEDSIDDTFLPVPEyINQSVPK(R)	**1068**	**3.8**	**8.1**	1160.210	**3**
(K)RPAGSVQNPVyHNQPLNPAPSR(D)	**1086**	**3.4**	**11.7**	827.075	**3**
(K)GSHQISLDNPDyQQDFFPK(E)	**1148**	**6.0**	**10.1**	1158.510	**2**
(K)GSTAENAEyLR(V)	**1173**	**2.3**	**8.8**	645.775	**2**

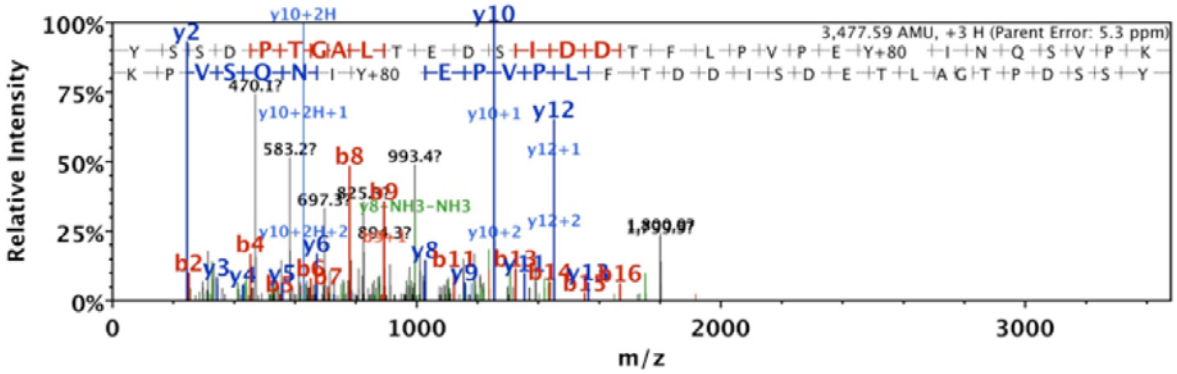
Residues in parentheses are deduced, and shown to indicate the peptides as tryptic fragments. Phosphotyrosine is denoted by non-capital y.


A
T = 0
T = 10
IB: EGFR pY1068
170
130
95
72
55
43
34
B
ShcD-GFP
EGFR pY1068
DAPI
Composite
COS-1 + EGF
HEK 293T + MuSK


COS-1
HEK 293
ShcD-GFP
GFP
ShcD-GFP
GFP
IB: p-Tyr
180
IB: EGFR
180
IB: FLAG
135
IB: GAPDH
35

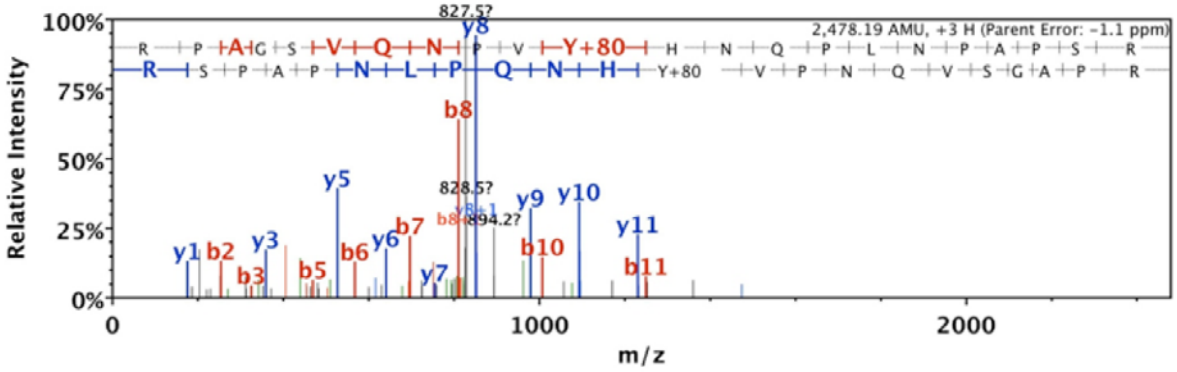
A

pY1068



B

pY1086



C

pY1173