SUPPLEMENTARY INFORMATION

Zheng et al. Temporal regulation of EGF signaling networks by the scaffold protein Shc1

SUPPLEMENTARY TEXT

EGF concentration selection and optimization

EGF levels vary greatly in various bodily fluids, from low (1–5 ng/ml) in plasma, serum, and saliva to medium (5–50 ng/ml) in tears, follicular fluid, sperm, and seminal plasma, to high (50–500 ng/ml) in bile, urine, milk, and prostate fluid[1-3]. To select an optimal EGF concentration for Shc1 signaling analysis by sMRM, we stimulated Rat-2 fibroblasts with increasing amounts of EGF and quantified the levels of Shc1 phosphopeptides and Shc1-interacting proteins by sMRM. The results confirmed that the measured Shc1-interaction network is EGF-dependent. In general, both Shc1 phosphorylation and levels of interacting proteins showed typical Michaelis–Menten kinetics with a saturating concentration of 100 ng/ml (Supplementary Fig. 1), which is on the high end of physiological concentrations. Different concentrations of EGF can result in different kinetics of protein interactions and physiological responses. Indeed, stimulating cells with 1 ng/ml or 10 ng/ml EGF resulted in slower rates of protein-protein interactions and phosphorylation, yet the sequence of Shc1 phosphorylation and the nature and kinetics of Shc1 protein interactions were very similar to those observed upon

stimulation with 100 ng/ml (data not shown). However, stimulating cells with very low EGF doses only allowed us to detect a subset of Shc1-interacting proteins, likely because the signal intensity for some binders was out of the sensitivity range of our sMRM assay. Thus, to ensure reproducibility across different biological repeats as well as the linearity of our quantification, we chose to use 100 ng/ml EGF in all subsequent studies.

Reference

1 Westergaard, L. G. *et al.* Epidermal growth factor in small antral ovarian follicles of pregnant women. *J Endocrinol* **127**, 363-367 (1990).

2 Richards, R. C. *et al.* Epidermal growth factor receptors on isolated human placental syncytiotrophoblast plasma membrane. *Placenta* **4**, 133-138 (1983).

3 Grau, M. *et al.* Relationship between epidermal growth factor in mouse submandibular glands, plasma, and bile: effects of catecholamines and fasting. *Endocrinology* **135**, 1854-1862 (1994).

Rationale for using Rat-2 fibroblasts as the model system

Although Rat-2 fibroblasts are immortalized, they are still considered to be phenotypically normal, non-transformed cells for studying receptor tyrosine kinase signaling. They have previously been shown to respond to EGF simulation[4-8]. We

also chose Rat-2 fibroblasts for a number of technical reasons. Firstly, Rat-2 fibroblasts are highly transfectable and easy to handle in large-scale tissue culture. More importantly, Rat-2 fibroblasts provide much higher concentration of proteins compared to MEFs, allowing for a higher coverage of the Shc1 protein interaction network and better data quality for sMRM quantifications. Finally, the sMRM assay we have designed using Rat-2 fibroblasts can be easily applied to mouse cell lines due to high sequence homology between rat and mouse, facilitating later studies in the various MEF cell lines that we have constructed.

References

4 Nicholson, P.R. *et al.* ShcA tyrosine phosphorylation sites can replace ShcA binding in signalling by middle T-antigen. *EMBO J.* **20**:6337-6346 (2001).

5 Wu, J. *et al.* Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3',5'-monophosphate. *Science* **262**:1065-1069 (1993).

6 Qiu, R.G.*et al.* An essential role for Rac in Ras transformation. *Nature* **374**:457-459 (1995).

7 Haley, J.D. et al. Analysis of mammalian fibroblast transformation by normal and mutated human EGF receptors. *Oncogene* **4**:273-283 (1989).

8 Kim, B.C. *et al.* Rac GTPase activity is essential for EGF-induced mitogenesis. *Mol Cells* **8**:90-95 (1998).

Choosing Shc1 normalizing peptides for data normalization

The abundance of proteins and phosphopeptides measured by sMRM was normalized to the protein level of Shc1 using five Shc1 normalizing peptides as references. Ideally, peptides without any post-translational modifications should be

used as references for normalization. However, due to limited availability of such peptides, we used Shc1 peptides with Met, Trp, or His residues, which may undergo oxidation (peptide 1, 2, and 5 in Supplementary Fig. 2). To increase the accuracy of sMRM quantification, we measured the major oxidized forms, as well as unmodified forms of these peptides in all experiments. For data normalization, we then used the summed extracted ion chromatograms (XICs) for any given Shc1 peptide as the reference.

Phosphorylation on Shc1 Y239/240 motif

We only detected the Y239/240 motif of Shc1 as a singly phosphorylated species in our survey scans due to the weak ion intensity of the corresponding peptide. In order to determine if this motif can be doubly phosphorylated, we specifically targeted the doubly-phosphorylated form by sMRM using electronically predicted MS properties and confirmed the presence of both singly and doubly phosphoforms only in EGF-stimulated samples. This observation may represent different activation states, as indicated by differentially phosphorylated forms of Shc1 during signal transduction. The temporal profile of the doubly phosphorylated Y239/240 site is similar to that of the singly phosphorylated form, although higher background interference was observed due to low signal to noise ratio. Therefore, the subsequent analyses were conducted on the singly phosphorylated Y239/240 motif (termed 1pY239/240).

Time control of immunoprecipitation experiments

Analysis of signaling complexes by mass spectrometry typically relies on immunoprecipitations, as is the case for our study. Given the dynamic nature of protein interactions, proteins often associate and disassociate from a complex during isolation *in vitro*, making the final composition of immunoprecipitates dependent on the amount of time the proteins in the complex had to exchange during cell lysate processing. As a result, there may be a subset of proteins that are lost or gained during our analysis. However, this issue should not affect our comparison between samples since each set of samples were processed at the same time under the same conditions. To further ensure consistency and minimize potential artifacts, we implemented several quality control steps in our experimental protocol including a defined 6-hour antibody incubation, timed buffer washes and a consistent amount of antibody.

Technical and biological replicates

For each MRM quantification experiment, we used 2-3 technical replicates, depending on the amount of available sample. We also performed a minimum of three biological replicates for each experiment. The error bars shown in the results were standard deviations from technical replicates within one biological experiment.

SUPPLEMENTARY TABLES

Supplementary Table 1

EGF-induced phosphorylation in Shc1-mediated signaling networks. Phosphorylated residues are highlighted in red. Novel sites are marked by asterisks.
Residue numbers are based on Rat sequence.

Protein	Residue	Position	Sequence	Novel	Binding partners	Upstream kinase
Shc1	Serine	29	HGSFVNKPTR		Ptpn12[1]	
Shc1	Threonine	214	LVTPHDR	*	Unknown	
Shc1	Tyrosine	313	ELFDDPSYVNIQNLDK		Grb2[2]	
Shc1	Tyrosine	239 /240	MAGFDGSAWDEEEEEPPDHQYYNDFPGK		Grb2[2]	
Shc1	Serine	335	QAGGGAGPPNPSLNGSAPR	*	Unknown	
Egfr	Tyrosine	1110	RPAGSVQNPVYHNQPLHPAPGR		Grb2[3,5]	
Egfr	Threonine	693	ELVEPLTPSGEAPNQAHLR		Unknown[4]	Erk[4]
Egfr	Tyrosine	1172	GSHQMSLDNPDYQQDFFPK		Shc1[5]	Src[6]
Egfr	Tyrosine	1196	GPTAENAEYLR		Shp1[7]	
Erbb2	Tyrosine	1200	DVFAFGGAVENPEYLVPR		Crk[8]	Src[6]
Gab1	Serine	277	VTSVSGESGLYNLPR		Unknown	
Gab1	Serine	293	SYSHDVLPK		Unknown[9]	
Gab1	Threonine	414	SNTISTVDLNK		Unknown[9]	
Gab1	Serine	446	SSSLEGFHNQLK		Unknown	
Gab1	Tyrosine	654	QVEYLDLDLESGK		Ptpn11[10]	
Gab1	Tyrosine	686	VDYVVVDQQK		Ptpn11[10]	
Gab1	Serine	481	NVLAAGNVSGEELDENYVPMNPNSPPR		Unknown[9]	Erk[9]
Gab1	Serine	578	VKPAPLEIKPLSEWEELQAPVRSPITR		Unknown[9]	Erk[9]
Gab2	Serine	143	SSPAEFSSSQHLLR		Unknown	
Gab2	Tyrosine	632	KPSTSSVTSDEKVDYVQVDKEK		Shp2[9]	
Sos1	Serine	1196	TSISDPPESPPLLPPREPVR		Unknown[11]	Erk[11,12]
Sos1	Serine	1261	HLPSPPLTQEVDLHSIAGPPVPPR		Unknown[11]	Erk[11,12]

References for Supplementary table 1

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2. van der Geer P, *et al.* The Shc adaptor protein is highly phosphorylated at conserved, twin tyrosine residues (Y239/240) that mediate protein-protein interactions. *Curr Biol.* **6**, 1435-44 (1996).

3. Okutani T, *et al.* Grb2/Ash binds directly to tyrosines 1068 and 1086 and indirectly to tyrosine 1148 of activated human epidermal growth factor receptors in intact cells. *J Biol Chem.* **269**, 31310-4 (1994).

4. Xin Li, *et al.* ERK-dependent threonine phosphorylation of EGF receptor modulates receptor downregulation and signaling. *Cell Signal*. **20**, 2145–2155 (2009).

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7. Keilhack H, *et al.* Phosphotyrosine 1173 mediates binding of the protein-tyrosine phosphatase SHP-1 to the epidermal growth factor receptor and attenuation of receptor signaling. *J Biol Chem*. **273**, 24839-46 (1998).

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9. Lehr S, *et al.* Identification of major ERK-related phosphorylation sites in Gab1. *Biochem.* **43**, 12133-40 (2004).

10. Cunnick JM, *et al.* Phosphotyrosines 627 and 659 of Gab1 constitute a bisphosphoryl tyrosine-based activation motif (BTAM) conferring binding and activation of SHP2. *J Biol Chem.* **276**, 24380-7 (2001).

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Supplementary Table 2

List of identified EGF-dependent Shc1-interacting proteins targeted by sMRM.
Novel interactors are marked by asterisks.

Protein	Accession	MW	Function	Novel
Ap2a2	O94973	103960	Endocytosis	
Ap2s1	P53680	17018	Endocytosis	
Arhgef5	Q12774	176800	Rho GEF	*
Asap2	Q13191	109450	Arf GAP	*
Cblb	Q5VWQ8	131625	E3 ubiquitin-protein ligase	*
Dab2ip	O43150	111651	Ras GAP	*
Egfr	P00533	134277	Receptor tyrosine kinase	
Erbb2	Q13480	76616	Receptor tyrosine kinase	
Erbb3	Q9UQC2	74458	Receptor tyrosine kinase	
Errfi1	Q9H706	97186	Negative regulator of Egfr family protein	*
Fam59a	P62993	25206	Grb2 binding protein	*
Gab1	P04626	137911	Scaffold protein	
Gab2	P21860	148098	Scaffold protein	
Grb2	P42336	124284	Adaptor	
Lrrk1	P27986	83598	Serine/threonine kinase	*
Pik3ca	O00459	81545	Lipid kinase	
Pik3r1	P62136	37512	Lipid kinase subunit	
Pik3r2	Q05209	88106	Lipid kinase subunit	
Ppp1ca	Q9UJF2	128558	Serine/threonine phosphatase	*
Ppp1cg	Q86YV5	149311	Serine/threonine phosphatase	*
Ptpn11	Q9H792	193106	Tyrosine phosphatase	
Ptpn12	P29353	62822	Protein tyrosine phosphatase	
Rasal2	Q5TFU9	125290	Ras GAP	*
SgK223	Q8NEM2	75690	Rnd2 effector	*
SgK269	O15357	138599	Atypical protein kinase	*
Shcbp1	Q06124	68436	SH2 domain binding	
Ship2	Q07889	152464	Lipid phosphatase	
Sos1	Q07890	152979	Ras GEF	
Sos2	Q38SD2	225393	Ras GEF	

Supplementary Table 3

sMRM transitions used in this study. Q1 - mass-to-charge ratio (m/z) for the peptide ion selected in Q1; Q3 - m/z for the fragment ion selected in Q3; CE - collision energy; [Oxi] - oxidation; [Pho] - phosphorylation; [Dea] - deamination; y - y ion; b - b ion.

Protein	Peptide sequence	Q1	Q3	CE	RT	Fragment
Shc1	HLLLVDPEGVVR	673.9	1096.64	34.651	60.78	2y10
Shc1	HLLLVDPEGVVR	673.9	656.4	34.651	60.78	2y6
Shc1	HLLLVDPEGVVR	449.6	691.4	23.7824	60.78	3b6
Shc1	HLLLVDPEGVVR	449.6	656.4	23.7824	60.78	3y6
Shc1	H[Oxi]LLLVDPEGVVR	682.4	1096.64	35.0256	70.3	2y10
Shc1	H[Oxi]LLLVDPEGVVR	682.4	656.4	35.0256	70.3	2y6
Shc1	VMGPGVSYLVR	589.3	890.5	30.929	58.34	2y8
Shc1	VMGPGVSYLVR	589.3	947.53	30.929	58.34	2y9
Shc1	VM[Oxi]GPGVSYLVR	597.3	890.5	31.2812	53.265	2y8
Shc1	VM[Oxi]GPGVSYLVR	597.3	947.53	31.2812	53.265	2y9
Shc1	VEGGQLGGEEWTR	709.33	834.37	36.211	47.795	2y7
Shc1	VEGGQLGGEEWTR	709.33	947.45	36.211	47.795	2y8
Shc1	VEGGQLGGEEWTR	473.22	834.37	24.82168	47.795	3y7
Shc1	VEGGQLGGEEWTR	473.22	777.35	24.82168	47.795	3y6
Shc1	VEGGQLGGEEW[oxi]TR	717.33	850.37	36.56252	43.4	2y7
Shc1	VEGGQLGGEEW[oxi]TR	717.33	963.45	36.56252	43.4	2y8
Shc1	VEGGQLGGEEW[2oxi]TR	725.33	866.37	36.91452	44.4	2y7
Shc1	VEGGQLGGEEW[2oxi]TR	725.33	979.45	36.91452	44.4	2y8
Shc1	EAEALLQLNGDFLVR	844.9	1175.6	42.1756	79.5	2y10
Shc1	EAEALLQLNGDFLVR	844.9	821.43	42.1756	79.5	2y7
Shc1	EAEALLQLNGDFLVR	844.9	1061.58	42.1756	79.5	2y9
Shc1	ALDFNTR	418.72	537.28	23.424	42.23	2y4
Shc1	ALDFNTR	418.72	652.3	23.424	42.23	2y5
Shc1	HGS[Pho]FVNKPTR	408.21	615.36	21.961	22.76	3y5
Shc1	HGS[Pho]FVNKPTR	408.21	624.3	21.961	22.76	3b6-98
Shc1	HGS[Pho]FVNKPTR	408.21	510.3	21.961	22.76	3b5-98
Shc1	HGSFVNKPTR	381.54	642.3	20.8	20.175	3b6
Shc1	HGSFVNKPTR	381.54	528.3	20.8	20.175	3b5
Shc1	HGSFVNKPTR	571.81	948.53	30.16	20.175	2y8
Shc1	HGSFVNKPTR	571.81	1005.7	30.16	20.175	2y9
Shc1	ELFDDPSY[Pho]VNIQNLDK	663.96	844.45	33.21424	69.845	3y7
Shc1	ELFDDPSY[Pho]VNIQNLDK	663.96	1047.37	33.21424	69.845	3b8
Shc1	ELFDDPSY[Pho]VNIQNLDK	663.96	943.52	33.21424	69.845	3y8
Shc1	ELFDDPSY[Pho]VNIQNLDK	995.44	844.45	48.799	69.845	2y7
Shc1	ELFDDPSY[Pho]VNIQNLDK	995.44	1370.64	48.799	69.845	2y11
Shc1	ELFDDPSY[Pho]VNIQNLDK	995.44	943.52	48.799	69.845	2y8
Shc1	ELFDDPSYVNIQNLDK	955.46	1290.67	47.04	70.135	2y11
Shc1	ELFDDPSYVNIQNLDK	955.46	844.45	47.04	70.135	2y7
Shc1	ELFDDPSYVNIQNLDK	955.46	943.52	47.04	70.135	2y8
Shc1	ELFDDPSYVNIQNLDK	637.3	844.5	32.0412	70.135	3y7
Shc1	ELFDDPSYVNIQNLDK	637.3	943.52	32.0412	70.135	3y8
Shc1	ELFDDPSYVNIQNLDK	637.3	967.4	32.0412	70.135	3b8

Shc1	LVT[Pho]PHDR	459.22	607.27	25.205	22.53	2y5-98
Shc1	LVT[Pho]PHDR	459.22	706.34	25.205	22.53	2y6-98
Shc1	LVT[Pho]PHDR	459.22	524.26	25.205	22.53	2y4
Shc1	LVT[Pho]PHDR	306.48	353.67	17.48512	22.53	3y6-98
Shc1	LVT[Pho]PHDR	306.48	524.25	17.48512	22.53	3y4
Shc1	LVTPHDR	279.82	427.2	16.3	19.835	3y3
Shc1	LVTPHDR	419.23	524.26	23.446	19.84	2y4
Shc1	LVTPHDR	419.23	625.31	23.446	19.835	2y5
Shc1	QAGGGAGPPNPS[Pho]LN[Dea]GSAPR	893.42	595.3	44.311	38.41	2y12-98
Shc1	QAGGGAGPPNPS[Pho]LN[Dea]GSAPR	893.42	979.4	44.311	38.41	2y9
Shc1	QAGGGAGPPNPS[Pho]LN[Dea]GSAPR	893.42	881.4	44.311	38.41	2y9-98
Shc1	[PGQ]QAGGGAGPPNPS[Pho]LN[Dea]GSAPR	884.9	595.3	43.9356	42.43	2y12-98
Shc1	[PGQ]QAGGGAGPPNPS[Pho]LN[Dea]GSAPR	884.9	979.4	43.9356	42.43	2y9
Shc1	[PGQ]QAGGGAGPPNPS[Pho]LN[Dea]GSAPR	884.9	881.4	43.9356	42.43	2y9-98
Shc1	QAGGGAGPPNPSLN[Dea]GSAPR	853.39	1207.61	42.549	37.46	2y12
Shc1	QAGGGAGPPNPSLN[Dea]GSAPR	853.39	899.46	42.549	37.46	2y9
Shc1	QAGGGAGPPNPSLN[Dea]GSAPR	569.3	715.4	29	37.46	2y7
Shc1	QAGGGAGPPNPSLN[Dea]GSAPR	569.3	899.46	29	37.46	2y9
Shc1	[PGQ]QAGGGAGPPNPSLN[Dea]GSAPR	844.9	1207.61	42.1756	41.975	2y12
Shc1	[PGQ]QAGGGAGPPNPSLN[Dea]GSAPR	844.9	899.46	42.1756	41.975	2y9
Shc1	MAGFDGSAWDEEEEEPPDHQY[Pho]Y[Pho]NDFPGK	1140.78	448.25	54.2	66.1	3y4
Shc1	MAGFDGSAWDEEEEEPPDHQY[Pho]Y[Pho]NDFPGK	1140.78	301.2	54.2	66.1	3y3
Shc1	MAGFDGSAWDEEEEEPPDHQYY[Pho]NDFPGK	1114.11	829.4	53.021	66.16	3y13(2+)
Shc1	MAGFDGSAWDEEEEEPPDHQYY[Pho]NDFPGK	1114.11	301.2	53.021	66.16	3y3
Shc1	MAGFDGSAWDEEEEEPPDHQYY[Pho]NDFPGK	835.83	829.4	40.77652	66.16	4y13(2+)
Shc1	MAGFDGSAWDEEEEEPPDHQYY[Pho]NDFPGK	835.83	301.2	40.77652	66.16	4y3
Shc1	MAGFDGSAWDEEEEEPPDHQYYNDFPGK	1087.45	448.25	51.848	66.68	3y4
Shc1	MAGFDGSAWDEEEEEPPDHQYYNDFPGK	1087.45	789.4	51.848	66.68	3y13(2+)
Shc1	MAGFDGSAWDEEEEEPPDHQYYNDFPGK	815.83	448.25	38.89652	66.68	4y4
Shc1	MAGFDGSAWDEEEEEPPDHQYYNDFPGK	815.83	789.4	38.89652	66.68	4y13(2+)
Shc1	ESTTTPGQYVLTGLQSGQPK	1046.52	814.44	51.047	61.795	2y8
Shc1	ESTTTPGQYVLTGLQSGQPK	1046.52	915.49	51.047	61.795	2y9
Shc1	ESTTTPGQYVLTGLQSGQPK	698	814.44	34.712	61.795	3y8
Shc1	ESTTTPGQYVLTGLQSGQPK	698	915.49	34.712	61.795	3y9
Sos1	DGNSVIFSAK	519.27	565.33	27.848	45.86	2y5
Sos1	DGNSVIFSAK	519.27	751.43	27.848	45.86	2y7
Sos1	TEEGNPEVLR	572.29	613.37	30.181	35.32	2y5
Sos1	TEEGNPEVLR	572.29	913.47	30.181	35.315	2y8
Sos1	EYIQPVQLR	573.32	612.38	30.226	50.93	2y5
Sos1	EYIQPVQLR	573.32	740.44	30.226	50.93	2y6
Sos1	TFLTTYR	451.24	653.36	24.855	49.56	2y5
Sos1	TFLTTYR	451.24	540.28	24.855	49.555	2y4
Sos1	FEIPEPEPTEADR	765.3754	914.43	38.67652	55.32	2y8
Sos1	FEIPEPEPTEADR	765.3754	1140.5	38.67652	55.32	2y10
Sos1	TSISDPPES[Pho]PPLLPPREPVR	755.38	880.98	37.23672	56.55	3y15(2+)
Sos1	TSISDPPES[Pho]PPLLPPREPVR	755.38	831.99	37.23672	56.55	3y15-Pho(2+)
Sos1	TSISDPPES[Pho]PPLLPPREPVR	755.38	587.35	37.23672	56.55	3y10(2+)
Sos1	HLPSPPLTQEVDLHSIAGPPVPPR	639.35	565.35	31.1314	65.32	4y5
Sos1	HLPSPPLTQEVDLHSIAGPPVPPR	639.35	719.44	31.1314	65.32	4y8
Sos1	HLPS[Pho]PPLTQEVDLHSIAGPPVPPR	659.34	565.35	32.01096	67.56	4y5
Sos1	HLPS[Pho]PPLTQEVDLHSIAGPPVPPR	659.34	990.57	32.01096	67.56	4y10

Gab1	NVLAAGNVSGEELDENYVPMNPNSPPR	961.79	1009.49	46.31876	65.64	3y9
Gab1	NVLAAGNVSGEELDENYVPMNPNSPPR	961.79	505.24	46.31876	65.64	3y9(2+)
Gab1	NVLAAGNVSGEELDENYVPMNPNSPPR	961.79	1108.56	46.31876	65.64	3y10
Gab1	NVLAAGNVSGEELDENYVPMNPNS[Pho]PPR	988.45	1089.45	47.4918	67.29	3y9
Gab1	NVLAAGNVSGEELDENYVPMNPNS[Pho]PPR	988.45	545.77	47.4918	67.29	3y10(2+)
Gab1	NVLAAGNVSGEELDENYVPMNPNS[Pho]PPR	988.45	1188.52	47.4918	67.29	3y10
Gab1	VKPAPLEIKPLSEWEELQAPVRS[Pho]PITR	791.68	1094.56	37.83392	77.88	4y18(2+)
Gab1	VKPAPLEIKPLSEWEELQAPVRS[Pho]PITR	791.68	1109.64	37.83392	77.88	4y19-Pho(2+)
Gab1	SNT[Pho]ISTVDLNK	636.3	776.41	32.997	43.005	2y7
Gab1	SNT[Pho]ISTVDLNK	636.3	689.38	32.997	43.005	2y6
Gab1	SNTISTVDLNK	596.31	776.41	31.238	40.76	2y7
Gab1	SNTISTVDLNK	596.31	990.55	31.238	40.76	2y9
Gab1	SYS[Pho]HDVLPK	563.25	784.34	29.783	39.715	2b7-Pho
Gab1	SYS[Pho]HDVLPK	563.25	514.26	29.783	39.715	2y9-Pho(2+)
Gab1	SYS[Pho]HDVLPK	375.83	470.7	20.53652	39.715	3y8-Pho(2+)
Gab1	SYS[Pho]HDVLPK	375.83	389.2	20.53652	39.715	3y7-Pho(2+)
Gab1	SYSHDVLPK	349.16	479.7	19.5	33.765	3y8(2+)
Gab1	SYSHDVLPK	349.16	398.2	19.5	33.765	3y7(2+)
Gab1	VDY[Pho]VVVDQQK	636.79	617.33	33.019	36.835	2y5
Gab1	VDY[Pho]VVVDQQK	636.79	557.2	33.019	36.835	2b4
Gab1	VDYVVVDQQK	596.81	716.39	31.26	42.165	2y6
Gab1	VDYVVVDQQK	596.81	617.33	31.26	42.165	2y5
Gab1	QVEY[Pho]LDLDLESGK	794.88	876.45	39.97472	62.07	2y8
Gab1	QVEY[Pho]LDLDLESGK	794.88	1361.6	39.97472	62.07	2y11
Gab1	QVEY[Pho]LDLDLESGK	794.88	989.47	39.97472	62.07	2y9
Gab1	QVEYLDLDLESGK	754.88	876.45	38.21472	65.18	2y8
Gab1	QVEYLDLDLESGK	754.88	1281.64	38.21472	65.18	2y11
Gab1	QVEYLDLDLESGK	754.88	989.47	38.21472	65.18	2y9
Gab1	SSS[Pho]LEGFHNQLK	713.82	664.8	36.408	45.465	2y12-Pho(2+)
Gab1	SSS[Pho]LEGFHNQLK	713.82	1085.57	36.408	45.465	2y9
Gab1	SSS[Pho]LEGFHNQLK	713.82	577.8	36.408	45.455	2y10-Pho(2+)
Gab1	SSS[Pho]LEGFHNQLK	476.21	843.4	24.95324	45.455	3y7
Gab1	SSS[Pho]LEGFHNQLK	476.21	577.8	24.95324	45.455	3y10-Pho(2+)
Gab1	SSSLEGFHNQLK	449.55	586.8	23.7802	42.665	3y10(2+)
Gab1	SSSLEGFHNQLK	449.55	843.45	23.7802	42.665	3y7
Gab1	VTS[Pho]VSGESGLYNLPR	829.91	1192.6	41.51604	56.74	2y11
Gab1	VTS[Pho]VSGESGLYNLPR	829.91	919.49	41.51604	56.74	2y8
Gab1	VTSVSGESGLYNLPR	789.91	1192.6	39.75604	54.36	2y11
Gab1	VTSVSGESGLYNLPR	789.91	919.49	39.75604	54.36	2y8
Gab1	LTGDPDVLEYYK	706.846	1026.52	36.10122	56.2	2y8
Gab1	LTGDPDVLEYYK	706.846	1198.55	36.10122	56.2	2y10
Gab1	HVSISYDIPPTPGNTYQVPR	747.709	1031.52	36.8992	55.45	3y9
Gab1	HVSISYDIPPTPGNTYQVPR	747.709	915.46	36.8992	55.45	3b8
Gab1	TFPEGTLGQSSK	626.311	1003.5	32.55768	42.86	2y10
Gab1	TFPEGTLGQSSK	626.311	777.41	32.55768	42.86	2y8

Grb2	ESESAPGDFSLSVK	726.84	949.5	36.98096	53.64	2y9
Grb2	ESESAPGDFSLSVK	726.84	1020.54	36.98096	53.64	2y10
Grb2	FNSLNELVDYHR	753.87	1045.5	38.17	59.515	2y8
Grb2	FNSLNELVDYHR	753.87	1245.62	38.17	59.515	2y10
Grb2	FNSLNELVDYHR	502.91	590.26	26.12804	59.515	3y4
Grb2	FNSLNELVDYHR	502.91	689.33	26.12804	59.515	3y5
Grb2	DIEQVPQQPTYVQALFDFDPQEDGELGFR	1127.87	1147.54	53.626	94	3y10
Grb2	DIEQVPQQPTYVQALFDFDPQEDGELGFR	1127.87	585.29	53.626	94	3b5
Grb2	N[Dea]YVTPVNR	482.24	586.33	26.197	32.14	2y5
Grb2	N[Dea]YVTPVNR	482.24	685.4	26.197	32.14	2y6
Grb2	FGNDVQHFK	364.51	559.3	20.03844	35.675	3y4
Grb2	FGNDVQHFK	364.51	472.73	20.03844	35.675	3y8(2+)
Grb2	ATADDELSFK	548.76	924.43	29.145	46.62	2y8
Grb2	ATADDELSFK	548.76	853.39	29.145	46.62	2y7
Egfr	GPTAENAEY[Pho]LR	650.78	731.31	33.635	37.02	2y5
Egfr	GPTAENAEY[Pho]LR	650.78	845.36	33.635	37.02	2y6
Egfr	GPTAENAEYLR	610.8	765.39	31.875	39.89	2y6
Egfr	GPTAENAEYLR	610.8	894.43	31.875	39.89	2y7
Egfr	RPAGSVQNPVY[Pho]HNQPLHPAPGR	618.81	497.28	30.2	36.275	4y5
Egfr	RPAGSVQNPVY[Pho]HNQPLHPAPGR	618.81	810.42	30.2	36.275	4b8
Egfr	RPAGSVQNPVYHNQPLHPAPGR	598.81	497.28	29.34764	35.415	4y5
Egfr	RPAGSVQNPVYHNQPLHPAPGR	598.81	810.42	29.34764	35.415	4b8
Egfr	ELVEPLT[Pho]PSGEAPNQAHLR	713.34	835.45	35.387	51.32	3y7
Egfr	ELVEPLT[Pho]PSGEAPNQAHLR	713.34	1276.64	35.387	51.32	3y12
Egfr	ELVEPLTPSGEAPNQAHLR	686.7	835.45	34.215	50.775	3y7
Egfr	ELVEPLTPSGEAPNQAHLR	686.7	1276.64	34.215	50.775	3y12
Egfr	GSHQMSLDNPDY[Pho]QQDFFPK	778.67	970.42	39.26148	61.405	2b9
Egfr	GSHQMSLDNPDY[Pho]QQDFFPK	778.67	1152.48	39.26148	61.405	2y8
Egfr	GSHQM[Oxi]SLDNPDY[Pho]QQDFFPK	784	986.42	39.496	55.855	2b9
Egfr	GSHQM[Oxi]SLDNPDY[Pho]QQDFFPK	784	1152.48	39.496	55.855	2y8
Egfr	GSHQMSLDNPDYQQDFFPK	752	970.42	38.088	62.235	2b9
Egfr	GSHQMSLDNPDYQQDFFPK	752	1072.48	38.088	62.235	2y8
Egfr	YLVIQGDER	546.79	816.42	29.059	45.02	2y7
Egfr	YLVIQGDER	546.79	717.35	29.05876	45.02	2y6
Egfr	NLQEILIGAVR	613.4	870.56	31.9896	74.06	2y8
Egfr	NLQEILIGAVR	613.4	998.61	31.9896	74.06	2y9
Egfr	ELILEFSK	489.7766	623.33	26.55017	61.86	2y5
Egfr	ELILEFSK	489.7766	736.42	26.55017	61.86	2y6
Egfr	VLGSGAFGTVYK	599.8237	986.49	31.39224	54.08	2y10
Egfr	VLGSGAFGTVYK	599.8237	842.43	31.39224	54.08	2y8
Ptpn11	INAAEIESR	501.77	633.32	27.07788	38.075	2y5
Ptpn11	INAAEIESR	501.77	775.39	27.078	38.075	2y7
Ptpn11	FDSLTDLVEHYK	489.58	1004.5	25.542	70.235	3y8
Ptpn11	FDSLTDLVEHYK	489.58	788.43	25.542	70.235	3y6
Ptpn11	ESAAHDYTLR	388.18	667.34	21.07992	31.305	3y5
Ptpn11	ESAAHDYTLR	388.18	473.7	21.07992	31.305	3y8(2+)
Ptpn11	ESAAHDYTLR	581.8	667.34	30.5992	31.305	2y5
Ptpn11	ESAAHDYTLR	581.8	804.4	30.5992	31.305	2y6
Ptpn11	IQNTGDYYDLYGGEK	868.3885	944.43	43.20909	53.35	2y8
Ptpn11	IQNTGDYYDLYGGEK	868.3885	1279.54	43.20909	53.35	2y11
Ptpn11	IQNTGDYYDLYGGEK	868.3885	1107.49	43.20909	53.35	2y9

Ptpn11	ESQSHPGDFVLSVR	519.92	573.4	26.87648	52.69	3y5
Ptpn11	ESQSHPGDFVLSVR	519.92	474.3	26.87648	52.69	3y4
Sgk269	LIVSNFSQAK	553.81	880.45	29.368	48.06	2y8
Sgk269	LIVSNFSQAK	553.81	781.38	29.368	48.06	2y7
Sgk269	FGVDSWSDFR	608.28	912.38	31.764	69.355	2y7
Sgk269	FGVDSWSDFR	608.28	797.36	31.764	69.355	2y6
Sgk269	EVPHLTVADFVR	461.26	607.32	24.295	67.065	3y5
Sgk269	EVPHLTVADFVR	461.26	536.28	24.295	67.065	3y4
Sgk269	ERPVPSTANSISSLATLSIK	691.05	919.55	34.406	65.33	3y9
Sgk269	ERPVPSTANSISSLATLSIK	691.05	632.4	34.406	65.33	3y6
Sgk269	LAPEIITATQYK	674.38	937.6	34.673	55.025	3y8
Sgk269	LAPEIITATQYK	674.38	824.45	34.673	55.025	3y7
Ptpn12	SFDGNTLLNR	568.79	787.44	30.027	51.005	2y7
Ptpn12	SFDGNTLLNR	568.79	902.47	30.027	51.005	2y8
Ptpn12	TLLLEFQNESR	675.35	1022.49	34.715	64.545	2y8
Ptpn12	TLLLEFQNESR	675.35	909.41	34.715	64.545	2y7
Ptpn12	AIAQLFEK	460.26	735.4	25.251	56.985	2y6
Ptpn12	AIAQLFEK	460.26	536.31	25.251	56.985	2y4
Ptpn12	SSKPQELSSGDLK	688.3535	1073.54	35.28755	32.23	2y10
Ptpn12	SSKPQELSSGDLK	688.3535	1116.51	35.28755	32.23	2b11
Ptpn12	SSKPQELSSGDLK	459.2383	606.31	24.20649	32.23	3y6
Ptpn12	SSKPQELSSGDLK	459.2383	519.28	24.20649	32.23	3y5
Ptpn12	ESEGLITSGNDK	625.2963	621.28	32.51304	33.3	2y6
Ptpn12	ESEGLITSGNDK	625.2963	734.37	32.51304	33.3	2y7
Fam59a	IEIPVHYAGQFK	467.92	850.42	24.588	56.155	3y7
Fam59a	IEIPVHYAGQFK	467.92	713.36	24.588	56.155	3y6
Fam59a	SDFLLDPSR	525.27	587.31	28.112	58	2y5
Fam59a	SDFLLDPSR	525.27	700.4	28.112	58	2y6
Fam59a	DELTQSFHR	566.78	775.38	29.938	41.13	2y6
Fam59a	DELTQSFHR	566.78	674.34	29.938	41.13	2y5
Fam59a	DELTQSFHR	378.18	546.27	20.63992	41.13	3y4
Fam59a	DELTQSFHR	378.18	674.34	20.63992	41.13	3b6
Fam59a	SPFGSPSAEALSSR	696.8312	917.46	35.66057	49	2y9
Fam59a	SPFGSPSAEALSSR	696.8312	1061.52	35.66057	49	2y11
Fam59a	LLNAPPVPPR	537.3251	662.4	28.6423	44.78	2y6
Fam59a	LLNAPPVPPR	537.3251	847.46	28.6423	44.78	2y8
Arhgef5	SFLILPFQR	560.83	660.38	29.677	81.065	2y5
Arhgef5	SFLILPFQR	560.83	547.3	29.677	81.065	2y4
Arhgef5	LLLQNILK	477.8184	728.47	26.02401	71.73	2y6
Arhgef5	LLLQNILK	477.8184	841.55	26.02401	71.73	2y7
Arhgef5	SGELTALEFSVSPGLK	817.9302	963.51	40.98893	71.74	2y9
Arhgef5	SGELTALEFSVSPGLK	817.9302	1076.59	40.98893	71.74	2y10
Arhgef5	FLVFDHAPFSSIR	512.6	706.39	26.554	74.26	3y6
Arhgef5	FLVFDHAPFSSIR	512.6	638.33	26.554	74.26	3y11(2+)
Arhgef5	VYLPYVTNQTYQER	887.44	1039.48	44.047	60.01	2y8
Arhgef5	VYLPYVTNQTYQER	887.44	1398.6	44.047	60.01	2y11
Arhgef5	VYLPYVTNQTYQER	591.97	1039.48	30	60.01	3y8
Arhgef5	VYLPYVTNQTYQER	591.97	696.33	30	60.01	3y5
Errfi1	HLSYVVSP	451.2408	600.31	24.8546	46.9	2b5
Errfi1	HLSYVVSP	451.2408	699.37	24.8546	46.9	2b6

Errfi1	VVPDPNPPPPQSHR	512.93	721.39	26.56892	28.81	3y6
Errfi1	VVPDPNPPPPQSHR	512.93	915.49	26.56892	28.81	3y8
Errfi1	SHS[Pho]GPAGSFNKPAIR	402.19	456.29	21.69636	36.76	4y4
Errfi1	SHS[Pho]GPAGSFNKPAIR	402.19	698.43	21.69636	36.76	4y6
Errfi1	SHS[Pho]GPAGSFNKPAIR	402.19	584.39	21.69636	36.76	4y5
Sgk223	LAPEIVSASQYR	667.36	575.3	34.364	50.735	2y10(2+)
Sgk223	LAPEIVSASQYR	667.36	711.34	34.364	50.735	2y6
Sgk223	GSLNQDLHGSGEEPLVVQGLSSR	793.73	1055.62	38.924	59.65	3y10
Sgk223	GSLNQDLHGSGEEPLVVQGLSSR	793.73	746.42	38.924	59.65	3y7
Sgk223	AEPEVYER	496.7379	792.39	26.85647	31.57	y6
Sgk223	AEPEVYER	496.7379	695.33	26.85647	31.57	y5
Sgk223	LGGLYTQSLAR	589.8269	675.38	30.95238	51.26	2y6
Sgk223	LGGLYTQSLAR	589.8269	838.44	30.95238	51.26	2y7
Sgk223	SASFAFEFPK	565.7763	738.38	29.89416	68.45	2y6
Sgk223	SASFAFEFPK	565.7763	972.48	29.89416	68.45	2y8
Ppp1cg	AHQVVEDGYEFFAK	547.27	641.33	28.08	58.085	3y5
Ppp1cg	AHQVVEDGYEFFAK	547.27	535.3	28.08	58.085	3b5
Ppp1cg	AHQVVEDGYEFFAK	547.27	836.39	28.08	58.085	3b8
Ppp1cg	AHQVVEDGYEFFAK	820.3846	1204.55	41.09692	58.085	2y10
Ppp1cg	AHQVVEDGYEFFAK	820.3846	1303.63	41.09692	58.085	2y11
Ppp1cg	AHQVVEDGYEFFAK	820.3846	861.41	41.09692	58.085	2y7
Ppp1cg	EIFLSQPILLELEAPLK	977.08	1235.76	47.992	95.56	2y11
Ppp1cg	EIFLSQPILLELEAPLK	977.08	1450.8	47.992	95.56	2y13
Ppp1cg	EIFLSQPILLELEAPLK	651.72	799.47	32.67568	95.56	3y7
Ppp1cg	EIFLSQPILLELEAPLK	651.72	912.54	32.67568	95.56	3y8
Ppp1ca	LNLDSIIGR	500.79	545.34	27.035	67.69	2y5
Ppp1ca	LNLDSIIGR	500.79	600.35	27.035	67.69	2y6
Ppp1ca	LNLDSIIGR	500.79	773.45	27.035	67.69	2y7
Ppp1ca	NVQLTENEIR	608.32	761.38	31.766	43.62	2y6
Ppp1ca	NVQLTENEIR	608.32	874.46	31.766	43.62	2y7
Ppp1ca	NVQLTENEIR	608.32	1002.54	31.766	43.62	2y8
Ppp1ca	YPENFFLLR	599.813	809.46	31.39177	76.89	2y6
Ppp1ca	YPENFFLLR	599.813	938.51	31.39177	76.89	2y7
Asap2	GDDNTGENNIVQELTK	873.9351	1244.65	43.45314	61.345	2y11
Asap2	GDDNTGENNIVQELTK	873.9351	717.43	43.45314	61.345	2y6
Asap2	GDDNTGENNIVQELTK	873.9351	1058.59	43.45314	61.345	2y9
Asap2	ASIEIANESGETPLDIAK	929.5	656.41	45.898	61.155	2y6
Asap2	ASIEIANESGETPLDIAK	929.5	1030.55	45.898	61.155	2y10
Asap2	ASIEIANESGETPLDIAK	929.5	1159.58	45.898	61.155	2y11
Asap2	EIISEVQR	487.26	618.34	26.43944	38.35	2y5
Asap2	EIISEVQR	487.26	731.41	26.43944	38.35	2y6
Asap2	GVDLLQNLIK	556.8447	728.49	29.50117	76.87	2y6
Asap2	GVDLLQNLIK	556.8447	956.59	29.50117	76.87	2y8
Asap2	NTVAAIEEALDVDR	758.3818	946.44	38.3688	74.08	2y8
Asap2	NTVAAIEEALDVDR	758.3818	1201.61	38.3688	74.08	2y11
Gab2	GSGESASWSAESPGK	718.8296	675.34	36.6285	36.085	2y7
Gab2	GSGESASWSAESPGK	718.8296	948.44	36.6285	36.085	2y9
Gab2	TQALQNTMQEWTDVR	910.95	1165.53	45.0818	65	2y9
Gab2	TQALQNTMQEWTDVR	910.95	933.44	45.0818	65	2y7
Gab2	TQALQNTMQEWTDVR	910.95	676.4	45.0818	65	2y5

Gab2	TQALQNTM[Oxi]QEWTDVR	918.95	1295.57	45.4338	61	2y10
Gab2	TQALQNTM[Oxi]QEWTDVR	918.95	1181.57	45.4338	61	2y9
Gab2	TQALQNTM[Oxi]QEWTDVR	918.95	933.44	45.4338	61	2y7
Gab2	SS[Pho]PAEFSSSQHLLR	542.5887	840.48	27.8739	49.57	3y7
Gab2	SS[Pho]PAEFSSSQHLLR	542.5887	927.52	27.8739	49.57	3y8
Gab2	GSEIQPPPVNR	597.3207	515.27	31.28211	36.98	2b5
Gab2	GSEIQPPPVNR	597.3207	679.4	31.28211	36.98	2y6
Gab2	GSEIQPPPVNR	597.3207	807.45	31.28211	36.98	2y8
Sos2	TVQDVEER	488.2403	775.36	26.48257	23.48	2y6
Sos2	TVQDVEER	488.2403	647.3	26.48257	23.48	2y5
Sos2	DENSVIFAAK	547.2851	648.43	29.08054	48.315	2y6
Sos2	DENSVIFAAK	547.2851	735.44	29.08054	48.315	2y7
Sos2	LPGYSSAEYR	571.7789	712.34	30.15827	42.445	2y6
Sos2	LPGYSSAEYR	571.7789	932.42	30.15827	42.445	2y8
Sos2	GEQPISADLK	529.2847	743.44	28.28853	39.79	2y7
Sos2	GEQPISADLK	529.2847	533.31	28.28853	39.79	2y5
Sos2	VLDDAVELSQDHFK	539.276	874.45	27.72814	54.01	3y7
Sos2	VLDDAVELSQDHFK	539.276	1003.5	27.72814	54.01	3y8
Erbb2	DVFAFGGAVENPEYLVPR	990.5	1116.57	48.582	81.71	2y9
Erbb2	DVFAFGGAVENPEYLVPR	990.5	1215.64	48.582	81.71	2y10
Erbb2	DVFAFGGAVENPEYLVPR	660.67	873.48	33.06948	81.71	3y7
Erbb2	DVFAFGGAVENPEY[Pho]LVPR	687.32	953.45	34.24208	85.53	3y7
Erbb2	DVFAFGGAVENPEY[Pho]LVPR	687.32	727.35	34.24208	85.53	3y5
Erbb2	DVFAFGGAVENPEY[Pho]LVPR	1030.48	953.45	50.341	85.53	2y7
Erbb2	DVFAFGGAVENPEY[Pho]LVPR	1030.48	1067.49	50.341	85.53	2y8
Erbb2	DVFAFGGAVENPEY[Pho]LVPR	1030.48	765.36	50.341	85.53	2b8
Erbb2	GLQSLSPHDLSPLQR	549.9711	828.47	28.19873	56.805	3y7
Erbb2	GLQSLSPHDLSPLQR	549.9711	1062.59	28.19873	56.805	3y9
Erbb2	EILDEAYVM[Oxi]AGVGSPYVSR	691.34	765.41	34.41896	68	3y7
Erbb2	EILDEAYVM[Oxi]AGVGSPYVSR	691.34	921.5	34.41896	68	3y9
Erbb2	EILDEAYVMAGVGSPYVSR	686.01	765.41	34.18444	74.35	3y7
Erbb2	EILDEAYVMAGVGSPYVSR	686.01	921.5	34.18444	74.35	3y9
Erbb2	WM[Oxi]ALESILR	567.81	801.51	29.98364	75.6	2y7
Erbb2	WM[Oxi]ALESILR	567.81	730.44	29.98364	75.6	2y6
Erbb2	WMALESILR	559.81	801.51	29.63164	81.11	2y7
Erbb2	WMALESILR	559.81	730.44	29.63164	81.11	2y6
Erbb2	GMSYLEDVR	535.25	881.44	28.551	50	2y7
Erbb2	GMSYLEDVR	535.25	631.34	28.551	50	2y5
Ship2	TLSEVDYSPGPGR	689.3466	848.4	35.33125	42.655	2y8
Ship2	TLSEVDYSPGPGR	689.3466	1163.53	35.33125	42.655	2y11
Ship2	VDQLNLER	493.7644	772.41	26.72563	41.385	2y6
Ship2	VDQLNLER	493.7644	531.3	26.72563	41.385	2y4
Ship2	VDQLNLER	493.7644	778.46	26.72563	41.385	2y7
Ship2	NQNYLDILR	574.8148	792.49	30.29185	65.175	2y6
Ship2	NQNYLDILR	574.8148	906.52	30.29185	65.175	2y7
Ship2	TGIANTLGNK	494.7749	646.37	26.7701	35.675	2y6
Ship2	TGIANTLGNK	494.7749	717.31	26.7701	35.675	2y7
Ship2	LDVTLGDLTK	537.8024	747.42	28.66331	61.37	2y7
Ship2	LDVTLGDLTK	537.8024	846.49	28.66331	61.37	2y8

Dab2ip	LGSFSTAAEELAR	676.8701	947.49	34.78228	60.015	2y9
Dab2ip	LGSFSTAAEELAR	676.8701	860.45	34.78228	60.015	2y8
Dab2ip	NSYLGLVSLPAASVAGR	837.9865	928.55	41.87141	73.24	2y10
Dab2ip	NSYLGLVSLPAASVAGR	837.9865	728.43	41.87141	73.24	2y8
Dab2ip	LISASLFLR	510.316	793.45	27.4539	72.74	2y8
Dab2ip	LISASLFLR	510.316	635.38	27.4539	72.74	2y5
Shcbp1	GAGIEIYPGSK	546.29	551.28	29.03676	47.22	2y5
Shcbp1	GAGIEIYPGSK	546.29	793.41	29.03676	47.22	2y97
Shcbp1	LSTFTLANVK	547.32	893.51	29.08208	58.115	2y8
Shcbp1	LSTFTLANVK	547.32	792.46	29.08208	58.115	2y7
Shcbp1	TSAELFM[Oxi]K	469.73	754.39	25.66812	46.55	2y6
Shcbp1	TSAELFM[Oxi]K	469.73	841.42	25.66812	46.55	2y7
Shcbp1	TSAELFMK	463.73	738.39	25.40412	49.835	2y6
Shcbp1	TSAELFMK	463.73	825.42	25.40412	49.835	2y7
Shcbp1	TPFPRIIQTNELLFYER	713.06	727.38	35.37464	94.71	3y5
Shcbp1	TPFPRIIQTNELLFYER	713.06	840.46	35.37464	94.71	3y6
Shcbp1	LIENPLLR	484.3	741.43	26.3092	59.89	2y5
Shcbp1	LIENPLLR	484.3	498.34	26.3092	59.89	2y4
Ap2a2	GLAVFISDIR	545.81	603.35	29.016	73.07	2y5
Ap2a2	GLAVFISDIR	545.81	750.41	29.016	73.07	2y6
Ap2a2	VVHLLNDQHLGVVTAATSLITTLAQK	686.63	661.38	34.21172	89.24	4y6
Ap2a2	VVHLLNDQHLGVVTAATSLITTLAQK	686.63	774.47	34.21172	89.24	4y7
Ap2a2	NADVELQQR	536.77	673.36	28.61788	30.54	2y5
Ap2a2	NADVELQQR	536.77	887.45	28.61788	30.54	2y7
Ap2a2	FVNLFPEVK	546.81	846.47	29.05964	71.71	2y7
Ap2a2	FVNLFPEVK	546.81	619.34	29.05964	71.71	2y5
Ap2s1	FILIQNR	452.27	643.39	24.89988	53.15	2y5
Ap2s1	FILIQNR	452.27	530.3	24.89988	53.15	2y4
Ap2s1	LIEEVHAVVTVR	455.6	644.4	24.0464	51.36	3y6
Ap2s1	LIEEVHAVVTVR	455.6	781.47	24.0464	51.36	3y7
Pik3r2	GFLLALPAAVVTPEAASEAYR	716.07	993.48	35.50708	86.54	3y9
Pik3r2	GFLLALPAAVVTPEAASEAYR	716.07	1094.52	35.50708	86.54	3y10
Pik3r2	GFLLALPAAVVTPEAASEAYR	716.07	1193.59	35.50708	86.54	3y11
Pik3r2	VALQALGVADGGER	678.3777	760.37	34.84862	53.23	2y8
Pik3r2	VALQALGVADGGER	678.3777	944.48	34.84862	53.23	2y10
Pik3r2	DTPDGTFLVR	560.7935	692.43	29.67491	53.03	2y6
Pik3r2	DTPDGTFLVR	560.7935	904.5	29.67491	53.03	2y8
Pik3r1	ALSEIFSHVLFR	473.59	758.42	24.83796	80.23	3y6
Pik3r1	ALSEIFSHVLFR	473.59	905.49	24.83796	80.23	3y7
Pik3r1	ISEIIDSR	466.75	819.42	25.537	39.3	2y7
Pik3r1	ISEIIDSR	466.75	732.38	25.537	39.3	2y6
Pik3r1	NESLAQYNPK	582.29	920.48	30.62076	37.59	2y8
Pik3r1	NESLAQYNPK	582.29	720.37	30.62076	37.59	2y6
Pik3r1	TQSSSNPAELR	595.29	960.47	31.19276	28.89	2y9
Pik3r1	TQSSSNPAELR	595.29	585.33	31.19276	28.89	2y 5
Pik3r1	TQSSSNPAELR	595.29	873.44	31.19276	28.89	2y8
Pik3ca	LINLTDILK	521.83	816.48	27.96052	79.49	2y7
Pik3ca	LINLTDILK	521.83	702.42	27.96052	79.49	2y6
Pik3ca	EAGFSYSHTGLSNR	509.24	647.35	26.40656	41.71	3y6
Pik3ca	EAGFSYSHTGLSNR	509.24	871.45	26.40656	41.71	3y8
Pik3ca	EAGFSYSHTGLSNR	509.24	546.3	26.40656	41.71	3y5

Erbb3	ELANEFTR	490.247	666.34	26.57087	42.83	2y5
Erbb3	ELANEFTR	490.247	737.37	26.57087	42.83	2y6
Erbb3	SPSQVQVADFGVADLLPPDDK	733.3868	912.48	36.26902	76.15	3b9
Erbb3	SPSQVQVADFGVADLLPPDDK	733.3868	684.38	36.26902	76.15	3y6
Erbb3	GESIEPLDPSEK	650.8104	785.42	33.63566	43.77	2y7
Erbb3	GESIEPLDPSEK	650.8104	914.45	33.63566	43.77	2y8
Erbb3	GESIEPLDPSEK	650.8104	460.26	33.63566	43.77	2y4
Erbb3	LAEIPDLLEK	570.8249	714.4	30.1163	67.66	2y6
Erbb3	LAEIPDLLEK	570.8249	956.52	30.1163	67.66	2y8
Cblb	ALEIAQNNLEVAR	720.8897	1014.52	36.71915	52.8	2y9
Cblb	ALEIAQNNLEVAR	720.8897	943.49	36.71915	52.8	2y8
Cblb	LAQLSENEYFK	671.33	916.4	34.53852	55.05	2y7
Cblb	LAQLSENEYFK	671.33	1029.49	34.53852	55.05	2y8
Cblb	EGFYLYPDGR	608.79	607.3	31.78676	60.16	2y5
Cblb	EGFYLYPDGR	608.79	720.39	31.78676	60.16	2y6
Lrrk1	GIRPVLGQPEEVQFHR	466.2553	523.35	24.51523	48.18	4b5
Lrrk1	GIRPVLGQPEEVQFHR	466.2553	587.31	24.51523	48.18	4y4
Lrrk1	LLVLTHGADPENYAVR	589.9923	848.44	29.95966	57.42	3y7
Lrrk1	LLVLTHGADPENYAVR	589.9923	1091.51	29.95966	57.42	3y10
Lrrk1	STLLEILQTGK	601.8624	788.48	31.48195	79.45	2y7
Lrrk1	STLLEILQTGK	601.8624	901.57	31.48195	79.45	2y8

Supplementary Table 4

sMRM transitions from αCasein spiked in as the internal control.

Protein	Peptide sequence	Q1	Q3	CE	RT	Fragment
aCasein_s2	ALNEINQFYQK	684.35	827.40	54.45	35.11	2y6
aCasein_s2	ALNEINQFYQK	684.35	940.49	35.111	54.45	2y7
aCasein_s1	VPQLEIVPNS[Pho]AEER	830.91	784.39	41.56	56.84	2y7-98
aCasein_s1	VPQLEIVPNS[Pho]AEER	830.91	882.34	41.56	56.86	2y7

Supplementary Table 5

Summary of Ser/Thr phosphorylation sites in Shc1 signaling network.

Data extracted from the phosphositeplus.org database at Cell Signaling Technology.
All proteins are human.

Protein	Accession	Evidence count for pS/T	Biological insight
AP2A2	O94973	S/T sites detected <5 times	No follow up
AP2S1	P53680	S/T sites detected <5 times	No follow up
ARHGEF5	Q12774	S1044; S1126 detected >5 times	No follow up
CBLB	Q13191	S476, S521, S525, S529, S672, S886 >5 times	No follow up
DAB2IP	Q5VWQ8	S702, S747, S971/2/3/4/8, S995, S1031, S1108 >5 times	S728 regulates molecular association[1]
ASAP2	O43150	S701, S722, S820, S822 > 5 times	No follow up
EGFR	P00533	T678, T693, S695, S991, S995, S1026, S1070, S1071, S1081, S1166 > 5times	T678, T693, S695, S991, S1070, S1071, S1190 have various functions
GAB1	Q13480	S266, S306, T316, S419, T638, S683, T684 > 5 times	No follow up
GAB2	Q9UQC2	S140, S250, S264, S322, T391, S404, S405, S472, S474, S543, S623, S625 > 5 times	S159, S210, T391, S623 regulate molecular interactions[2-4]
FAM59A	Q9H706	S473, S610, S614 > 5 times	No follow up
GRB2	P62993	> 5 times	No follow up
ERBB2	P04626	T701, S1054, S1151 > 5 times	T686 is implicated in receptor internalization[5-7]
ERBB3	P21860	S686 > 5 times	No follow up
PIK3CA	P42336	S312 > 5 times	No follow up
PIK3R1	P27986	T576, S608 > 5 times	S608 involved in "activation"[8]
PIK3R2	O00459	T468 > 5 times	No follow up
PPP1CA	P62136	T320 > 5 times	T320: Inhibitory site[9]
PPP1CC	P36873	T307	No follow up
PTPN12	Q05209	S39, S332, S435, S449, T509, T519, T569, S571, S603, S606, S608, S673 > 5 times	S39 implicated in inhibition[10]
RASAL2	Q9UJF2	S11, S16, T620, S736, S864, T941 > 5 times	No follow up
SGK223	Q86YV5	S490, S694, S743, S824 > 5 times	No follow up
SGK269	Q9H792	S281, S568, S572, T615, T1165, S1214, S1217 > 5 times	No follow up
SHC1	P29353	S29 > 5 times	S29 implicated in molecular association[11]
SHCBP1	Q8NEM2	S/T sites detected <5 times	No follow up
SHIP2	O15357	S132, S980, S1003, S1011, T1013, S1131, S1160 > 5 times	T958 involved in "phosphorylation" [12]
PTPN11	Q06124	S302, T547, T557, T568, S595 > 5 times	No follow up
SOS1	Q07889	S1082, S1134, S1137, S1161 > 5 times	Regulation of molecular association by S1132, S1167, S1178, S1193, S1197[13]
SOS2	Q07890	S/T sites detected <5 times	No follow up
LRRK1	Q38SD2	S360, T358 detected > 5 times	No follow up
ERRIF1	Q9UJM3	S249, 251, 273, 461	No follow up

References for Supplementary Table 5

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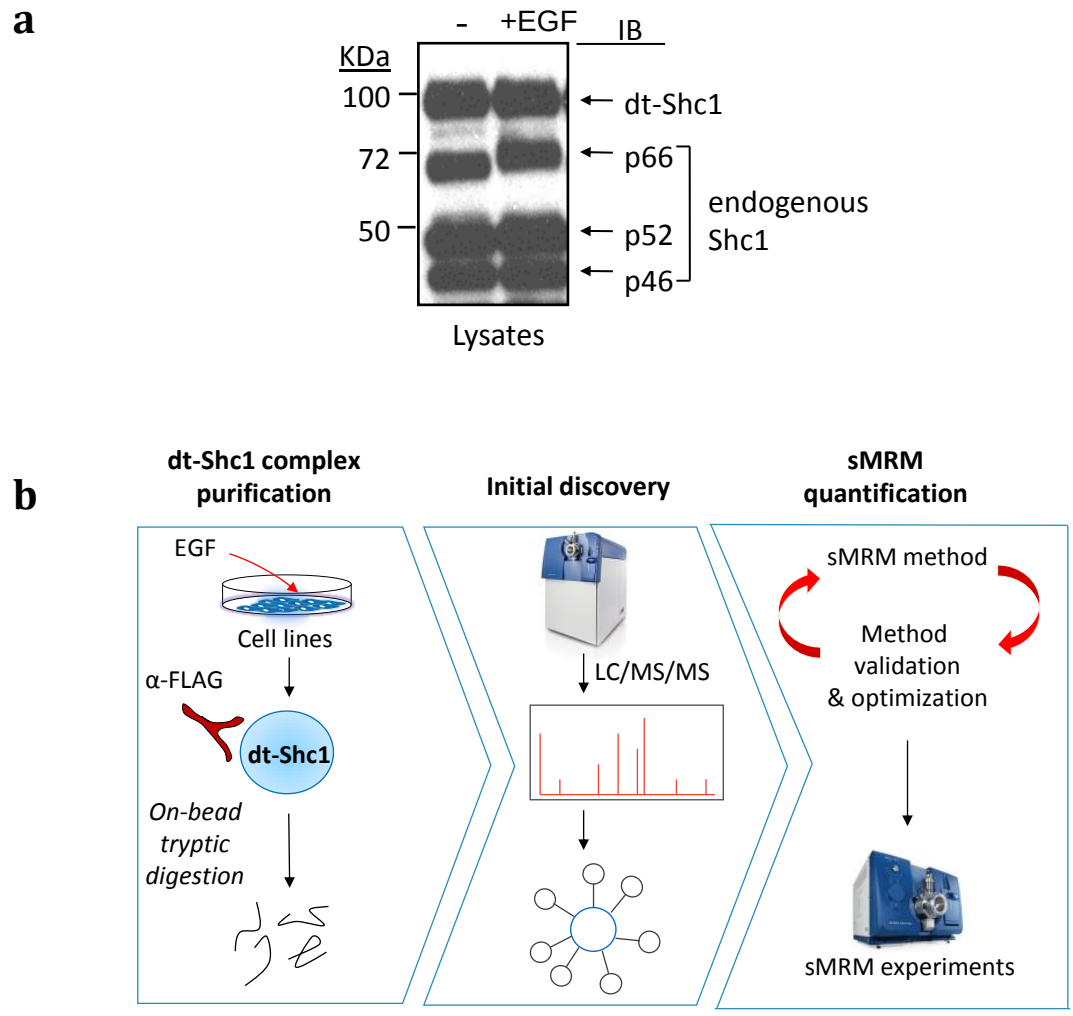
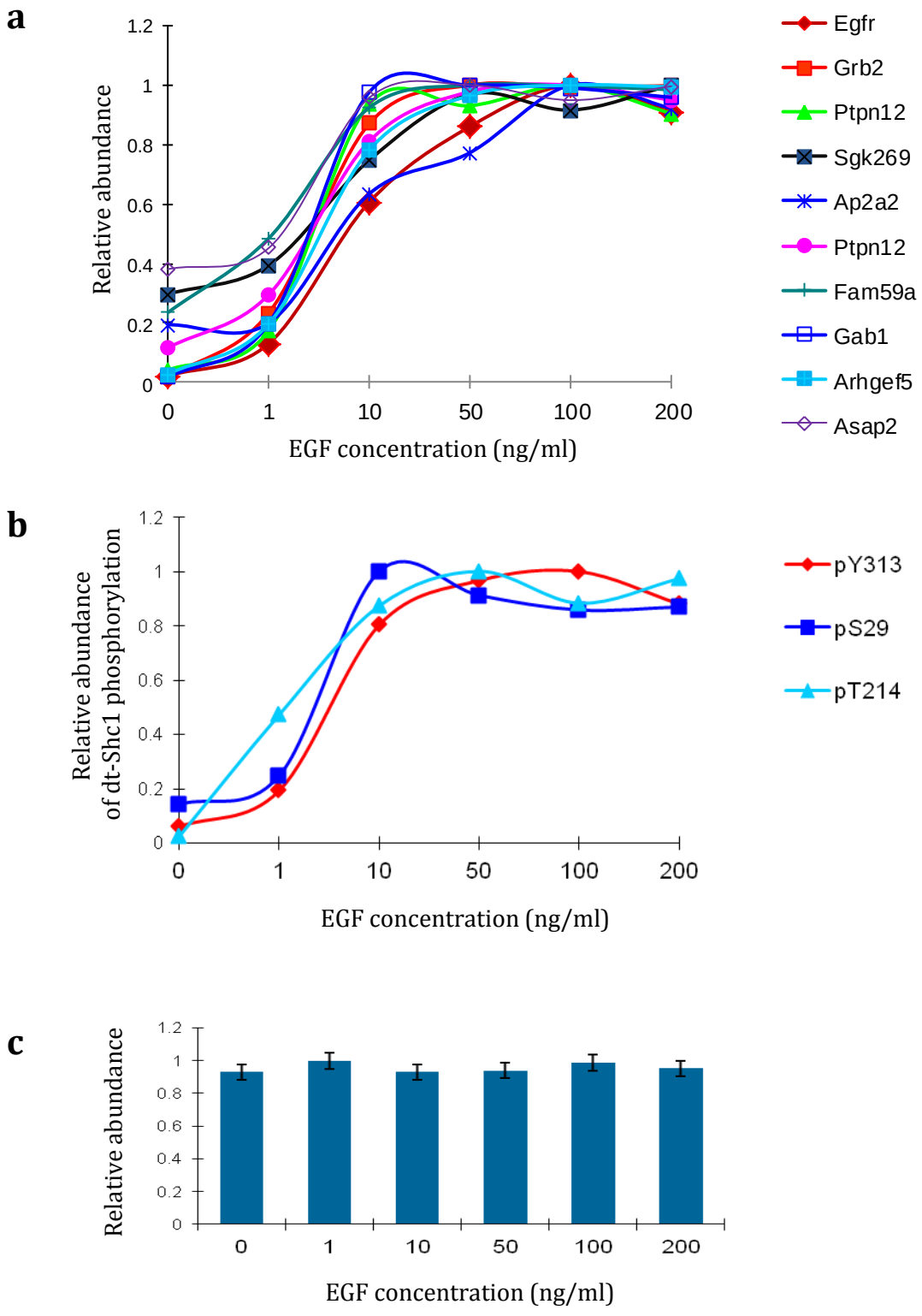
SUPPLEMENTARY FIGURES

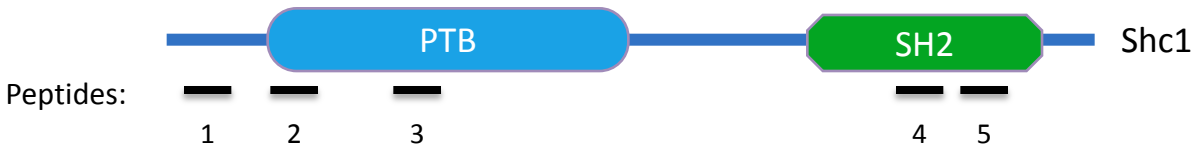


Supplementary Figure 1. Workflow for sMRM analysis. a, Expression level of dt-Shc1 in a stable Rat-2 fibroblast cell line. Lysates prepared from Rat-2 fibroblasts stably expressing dt-Shc1 were immunoblotted with anti-Shc1 antibodies. **b,** Workflow for sMRM analysis. dt-Shc1 was affinity purified from EGF-stimulated cells and digested with trypsin. The peptides were analyzed by LC/MS in discovery mode to identify a Shc1-based interaction network. This information was used to build an sMRM method, which was employed to quantify dynamic changes in the Shc1 network.



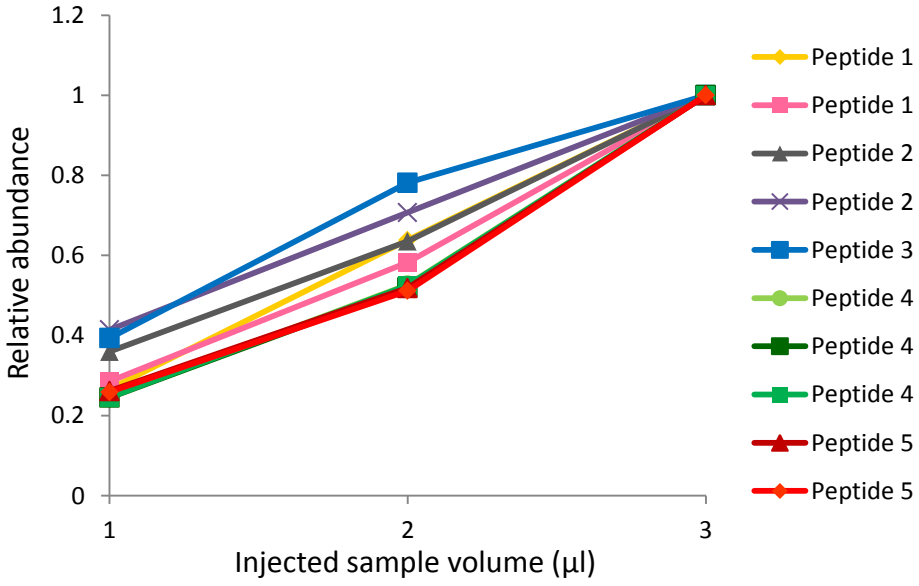
Supplementary Figure 2. EGF dose optimization. a-b, Cells were stimulated with increasing concentrations of EGF for 5 min. Interacting proteins and Shc1 phosphopeptides in purified dt-Shc1 complexes were quantified by sMRM. **c,** Normalized Shc1 levels. Error bars are +/- s.d. (n=3).

a

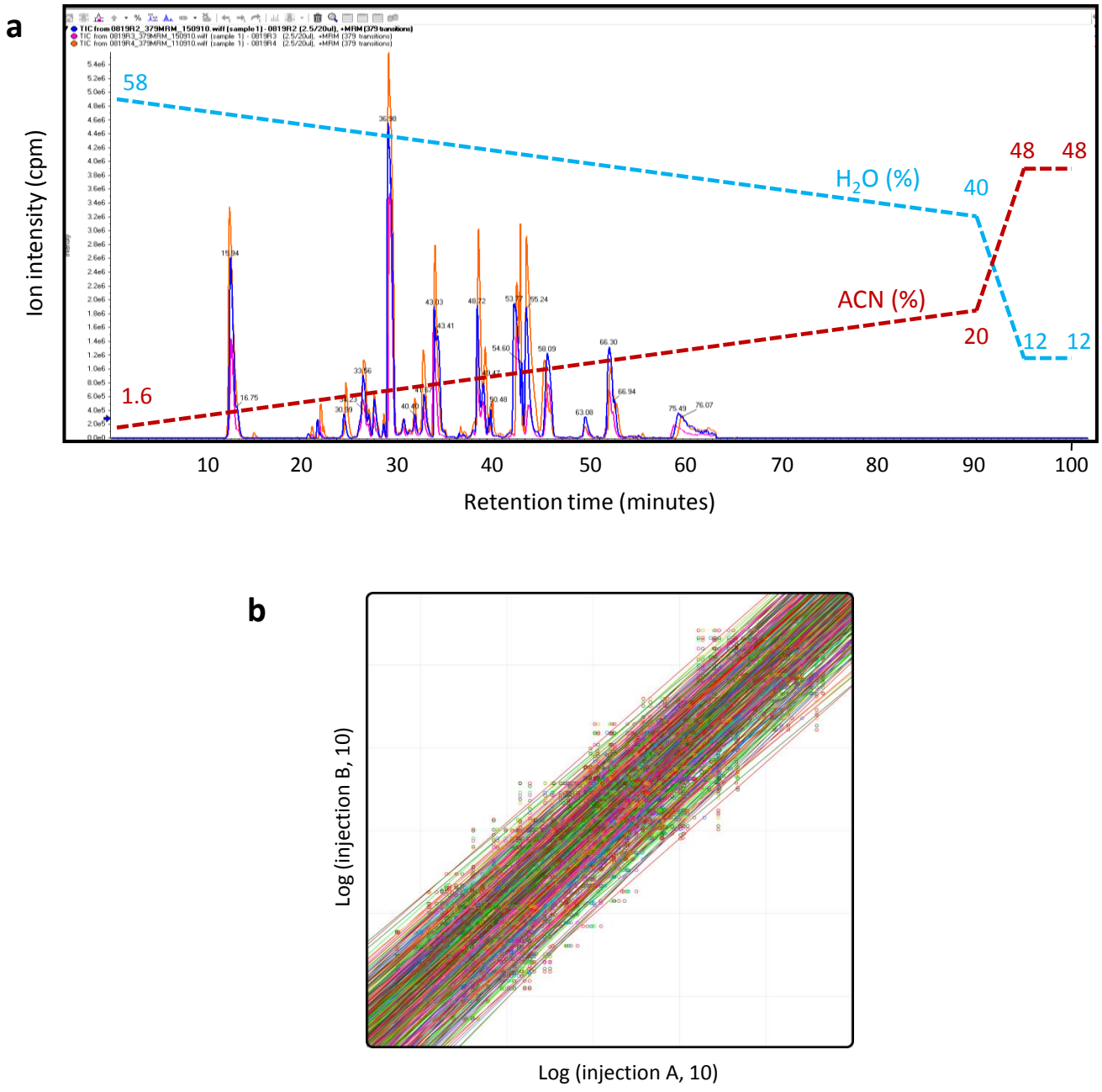


No.	Sequence	m/z
1	VEGGQLGGEEWTR	709.3
2	VMGPGVSYLVR	589.3
3	ALDFNTR	418.7
4	ESTTTPGQYVLTGLQSGQPK	1046.5
5	HLLLVDPEGVVR	673.9

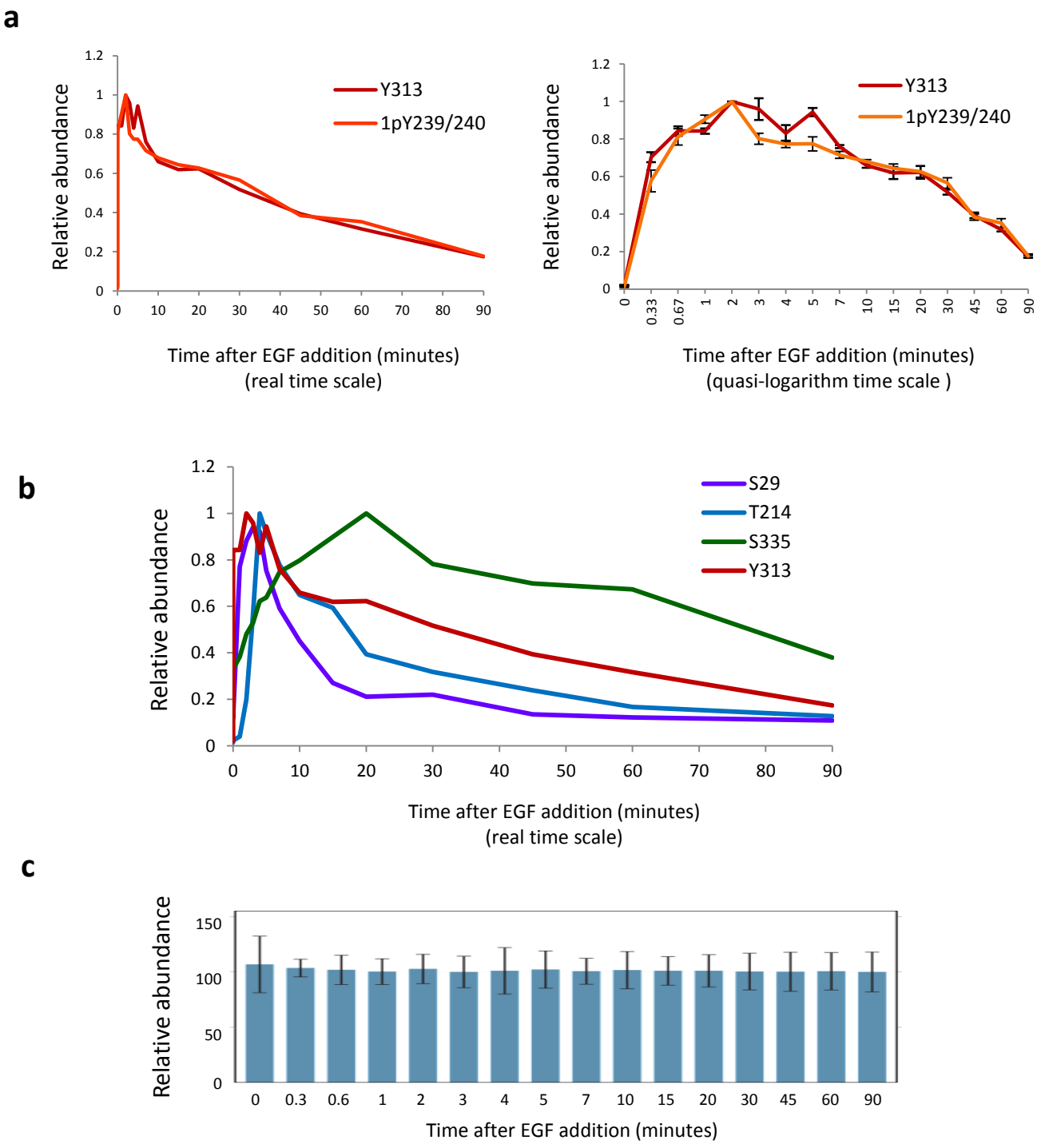
b



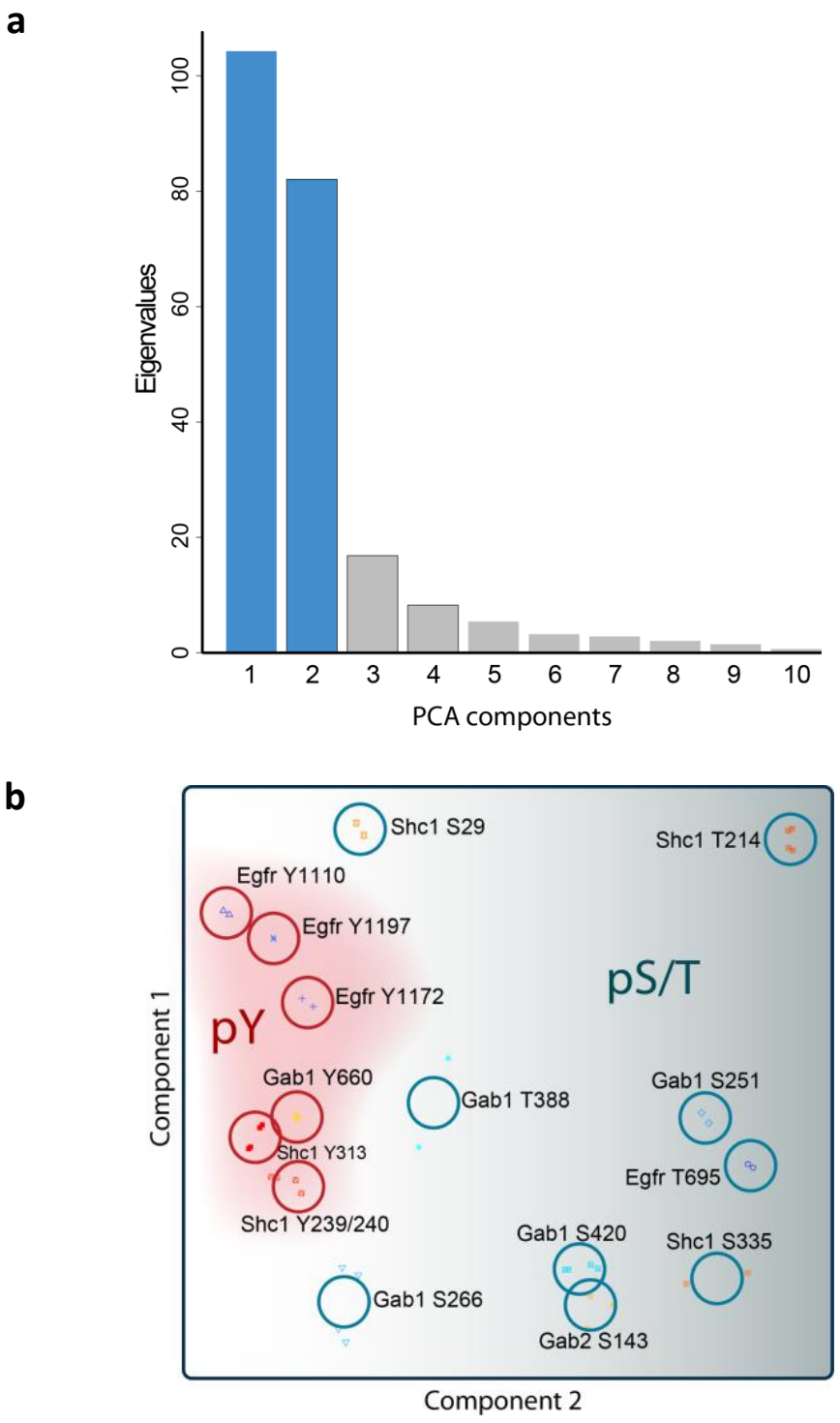
Supplementary Figure 3. Normalization peptides and linearity of sMRM assay for Shc1 complexes. **a,** Relative positions and sequences of dt-Shc1 peptides used for normalization. **b,** Linearity plot of dt-Shc1 normalizing peptides. Tryptic digested dt-Shc1 immunoprecipitates were prepared as described in **Fig. 1b** and the indicated volumes were injected. Relative abundance of dt-Shc1 normalizing peptides were measured by sMRM. dt-Shc1 peptides are numbered as in **a** and the transitions for each peptide are graphed.



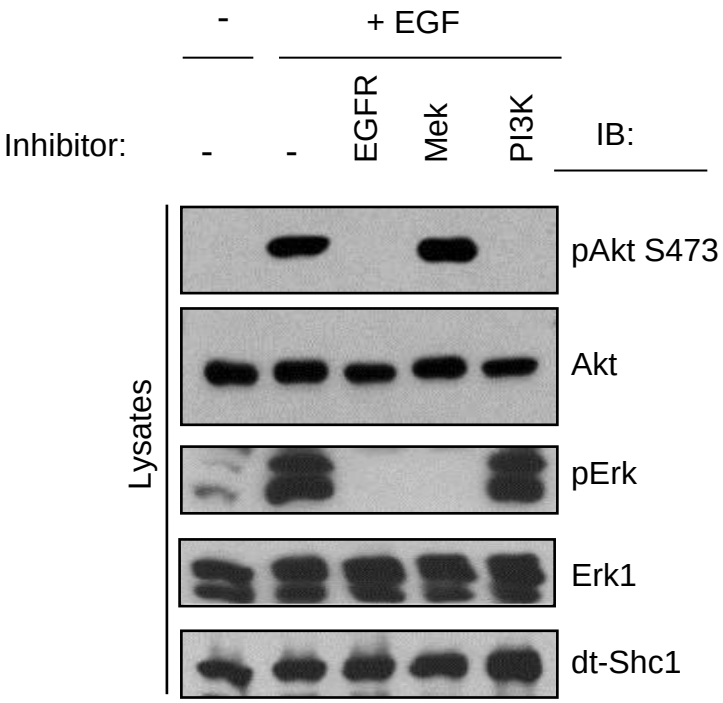
Supplementary Figure 4. Reproducibility of sMRM technical repeats. a, The overlay of three LC-sMRM chromatographs from three consecutive injections of the same biological sample (pink: injection a; blue: injection b; orange: injection c). Each chromatograph consists of the whole set of sMRM-targeted proteins for the Shc1 interactome. Each peak represents a detected peptide fragment (transition) from the Shc1 interactome. The peak areas for major peaks are labeled. The LC gradient for peptide elution is also shown by dotted lines (Blue: H_2O %; Red: ACN %). cpm=count per minute. **b,** Verification of the linear regression of sMRM data in **a**. The Log_{10} values of all data points (circles) from the first two injections (injection a and injection b) are plotted against each other. Each data point represents the ion intensity of a given peptide transition (peak area) quantified by the sMRM assay.



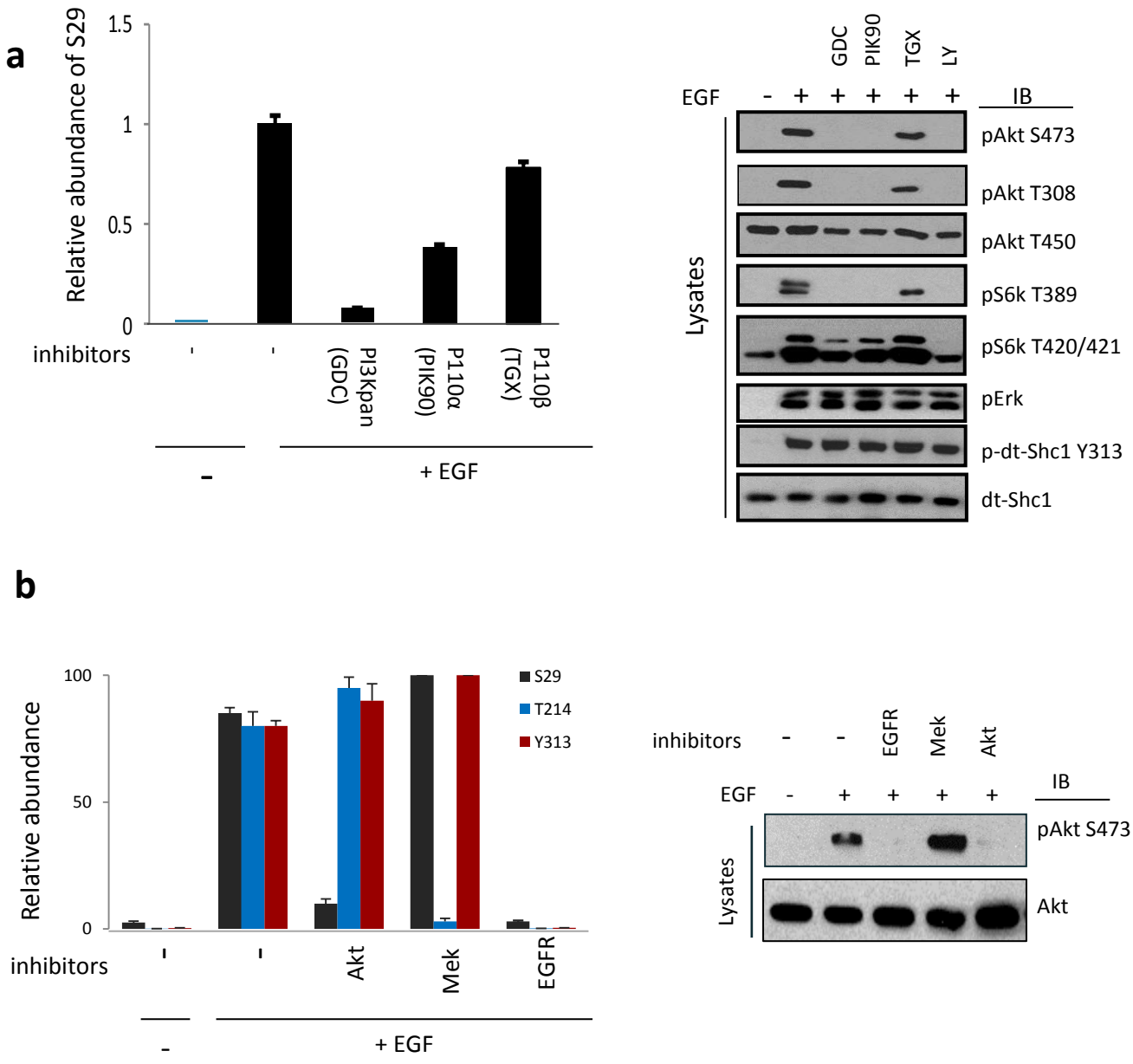
Supplementary Figure 5. Temporal profiles of dt-Shc1 phosphotyrosine sites after EGF stimulation. a, dt-Shc1 was affinity purified from EGF-stimulated fibroblasts. Dynamic phosphorylation on tyrosine 313 (Y313) and tyrosine 239/240 (1pY239/240, as singly phosphorylated form; see supplementary text for details) was measured by sMRM (left panel). Phosphorylation kinetics were plotted using a quasi-logarithm time scale to expand the early time points (right panel). **b,** Dynamics of all Shc1 phosphorylation sites plotted on a real time scale. **c,** Normalized dt-Shc1 levels. Results are representative of three independent biological repeats. Error bars are +/- s.d. from all transitions for each phosphopeptide from all technical repeats.



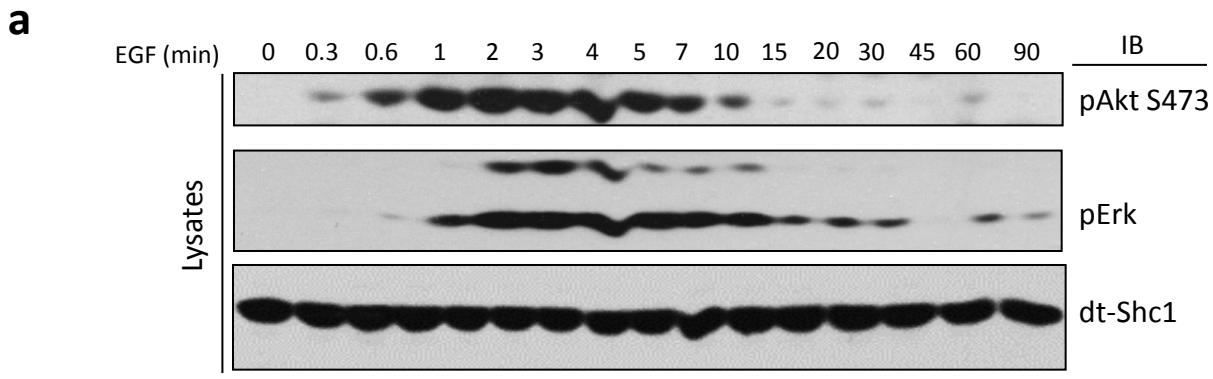
Supplementary Figure 6. Principle component analysis (PCA) of the dynamic profiles of phosphorylation sites from the Shc1-signaling network shown in Fig. 2b. a, Distribution of eigenvalues among PCA components, showing components 1 and 2 (in blue) with the highest contributions among all variances. **b,** PCA of analyzed phosphorylation sites in the Shc1-signaling network. The centre of the open circles marks the mean PCA values for each phosphorylation site. Open circles: red, tyrosine sites; blue, serine/threonine sites.

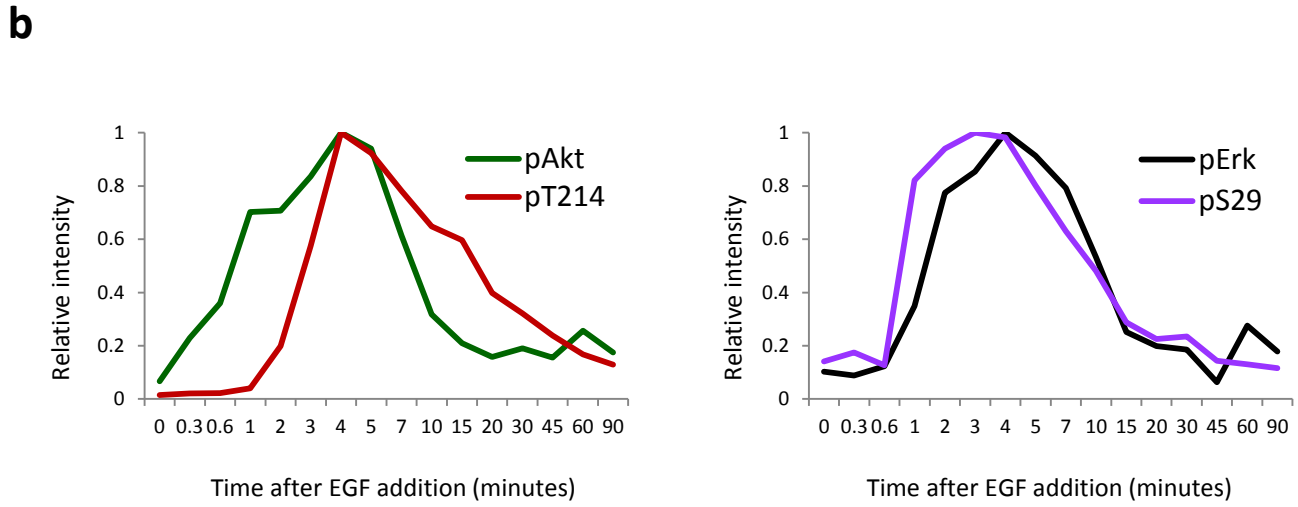


Supplementary Figure 7. Monitoring kinase inhibitor activity by immunoblotting. The inhibition efficiencies of indicated kinase inhibitors were monitored by immunoblotting for Erk and Akt (S473) phosphorylation. Inhibitor used for EGFR is AG1478; Mek is PD98059; PI3K is LY294002. Results are representative of three independent biological repeats.

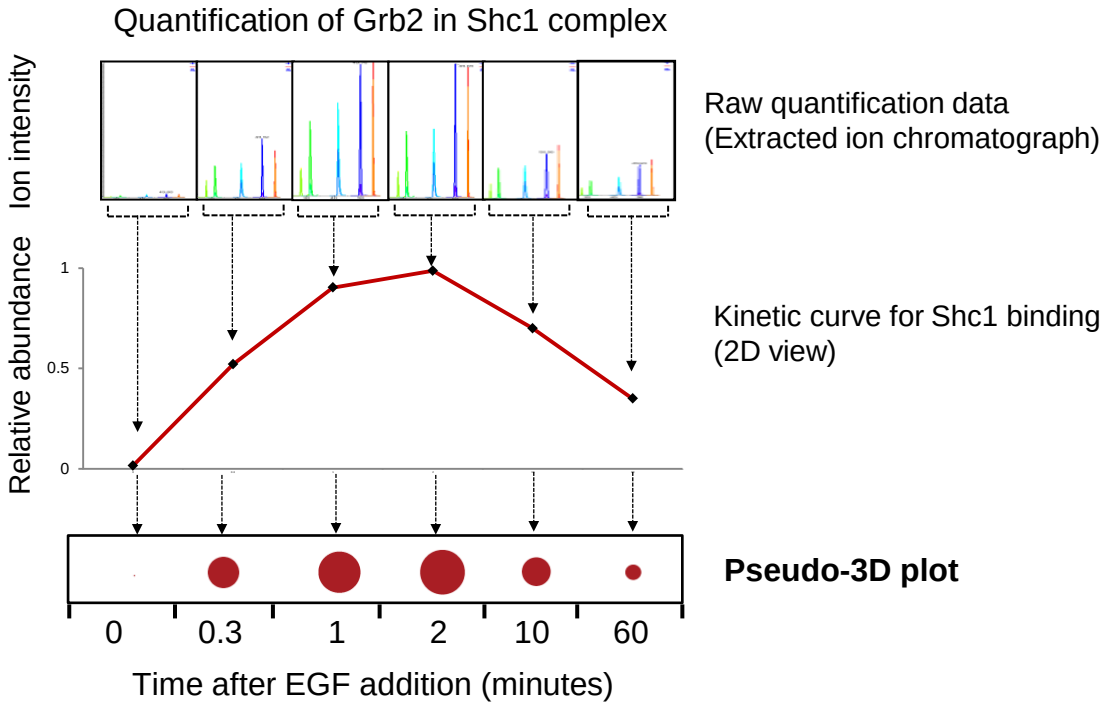


Supplementary Figure 8. EGF-induced phosphorylation of Shc1 Ser29 is sensitive to Akt inhibition and requires PI3K p110α. a, Effects of indicated inhibitors on dt-Shc1 S29 phosphorylation. Fibroblasts were pretreated for 20 min with isoform-specific PI3K inhibitors. Phosphorylation on dt-Shc1 S29 was quantified by sMRM (left panel). The efficiency of PI3K inhibition was monitored by measuring the phosphorylation on Akt and S6k using immunoblotting (right panel). **b,** Effects of an Akt-specific inhibitor (Akt inhibitor IV) on dt-Shc1 phosphorylation compared to Mek and EGFR inhibitors (left panel) as monitored by sMRM. The inhibition efficiency was monitored by immunoblotting for phospho-Akt (right panel). GDC: pan-PI3K inhibitor; PIK90: PI3K p110α isoform-specific inhibitor; TGX: PI3K p110β isoform-specific inhibitor, LY: pan-PI3K inhibitor (LY294002). To inhibit Akt, we used Akt inhibitor IV; Mek, PD98059; EGFR, AG1478. Results are representative of three independent biological repeats. Error bars are s.d. from all transitions for each phosphopeptide from all technical repeats. Data are the representative of three biological replicates.

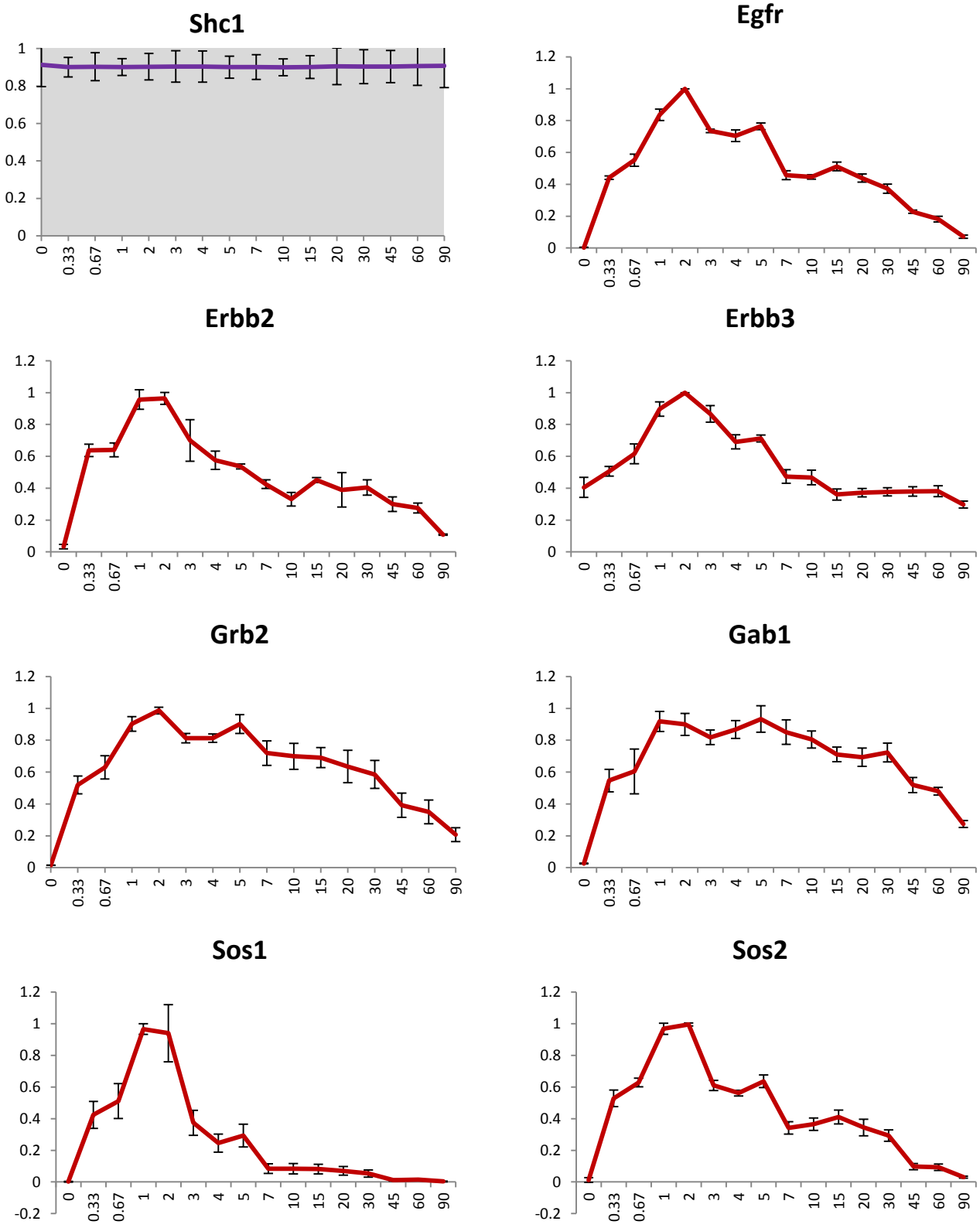




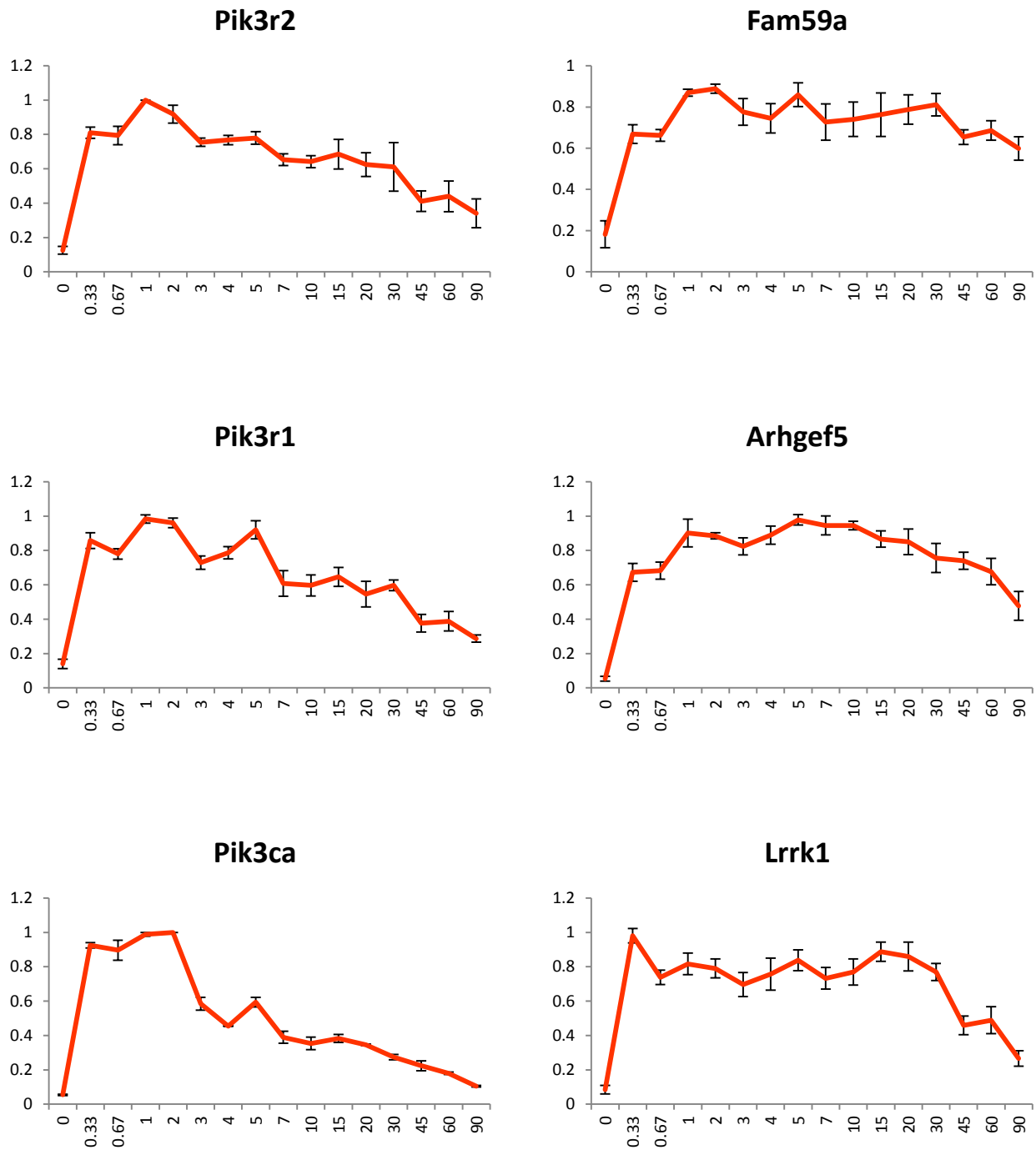
Supplementary Figure 9. Temporal profiles of Akt and Erk phosphorylation upon EGF stimulation. a, EGF-induced Akt and Erk phosphorylation kinetics were quantified by quantitative immunoblotting (Odyssey infrared imaging system, LI-COR Biosciences). **b,** Overlay of temporal phosphorylation profiles, showing the difference in kinetics of Akt activation (pAkt) versus Shc1 T214 phosphorylation (pT214), and between Erk activation (pErk) and Shc1 S29 phosphorylation (pS29) (compare with Fig. 2e).



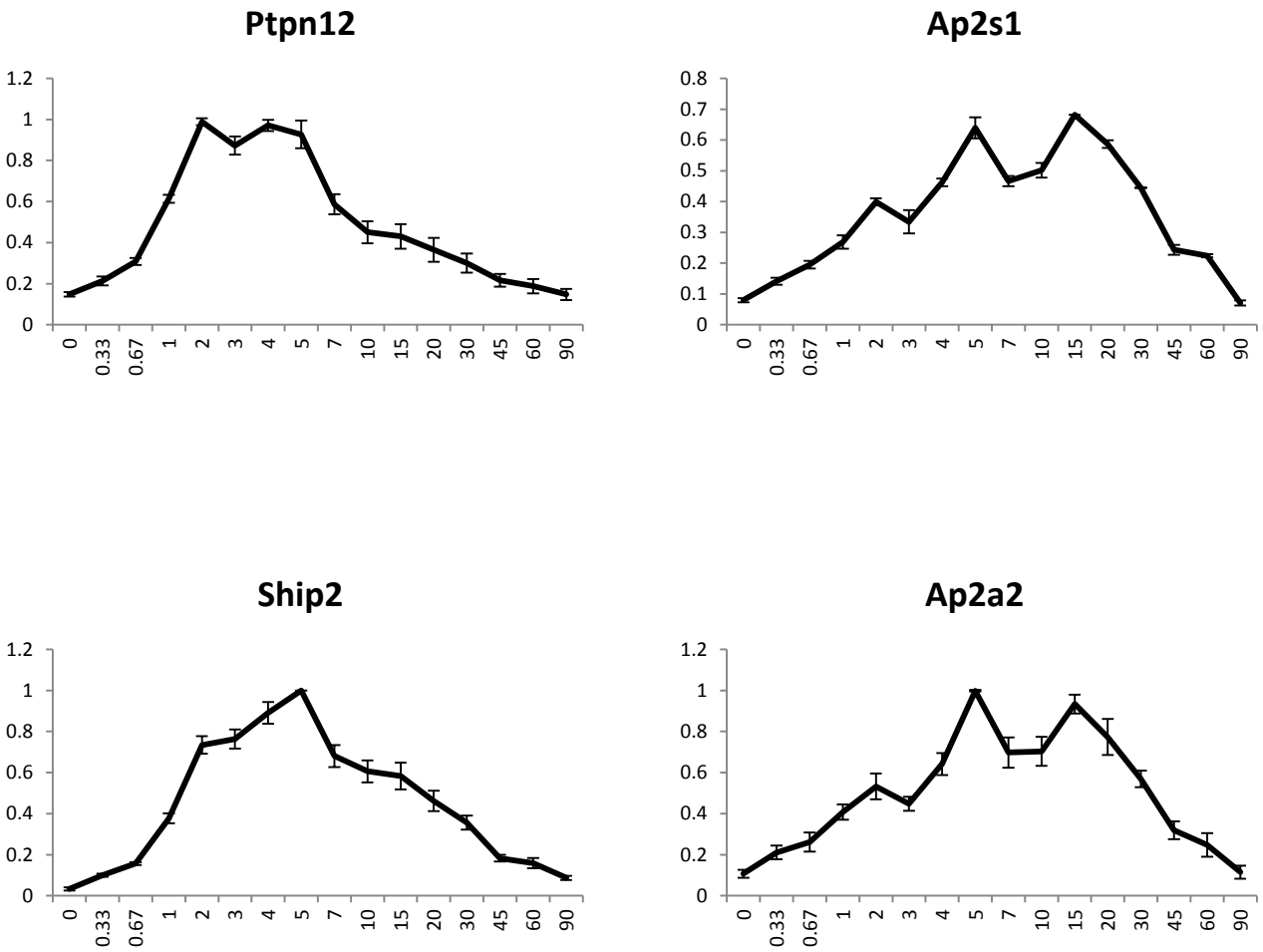
Supplementary Figure 10. Conversion of sMRM quantification data into pseudo-3D plots to generate temporal profiles for Shc1 binding proteins using Grb2 as an example. The abundance (ion intensity) of each Grb2 transition (peptide fragment) was first converted into relative abundance using the highest value of that transition across all time points as 1.0. The mean transitions from Grb2 were then plotted as a 2D kinetic curve, which was then converted into a pseudo-3D view. The sizes of the dots are proportional to the relative abundance of Shc1-associated Grb2 at the indicated time points.



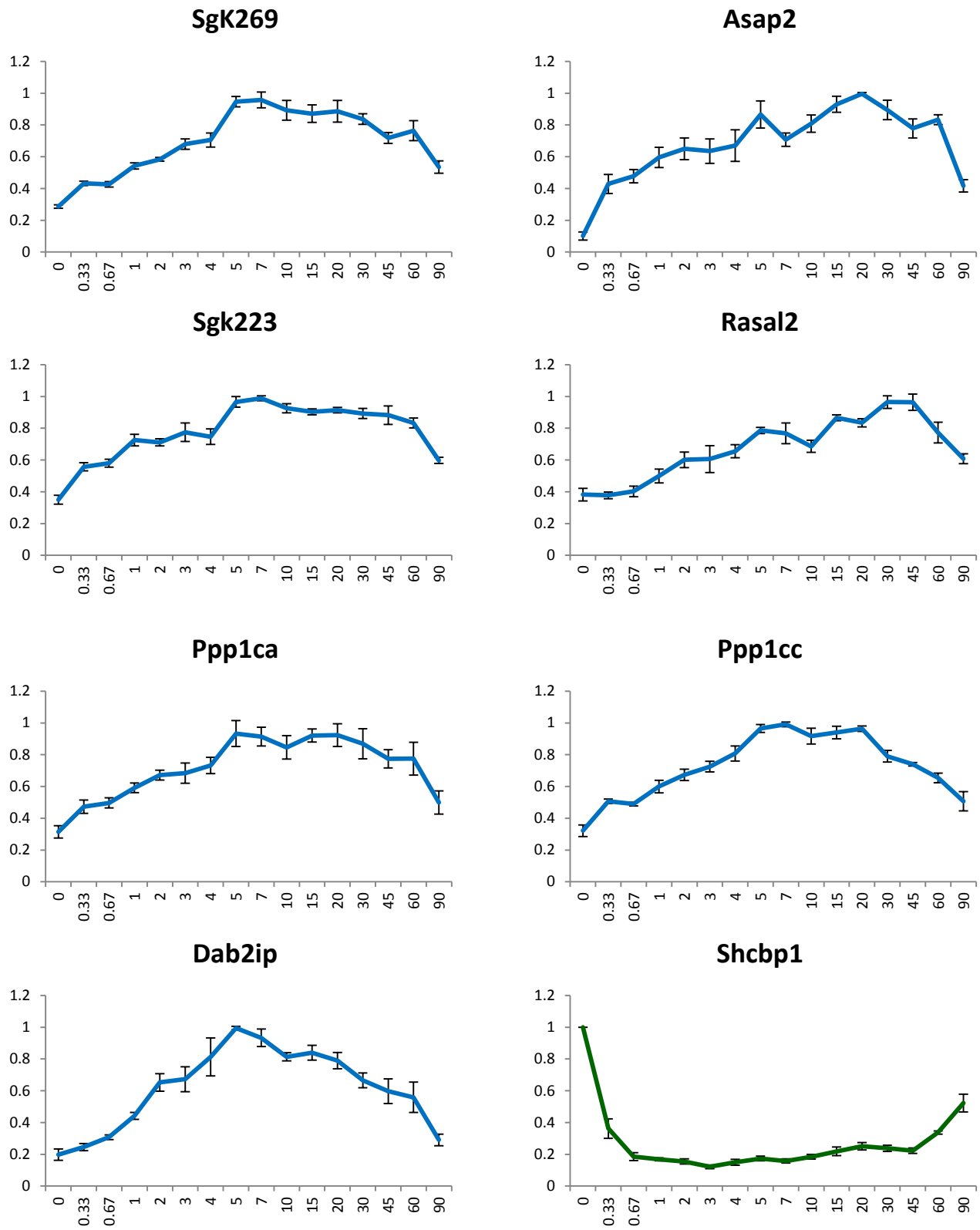
Supplementary Figure 11. Dynamic profiles of Cluster 1a Shc1-interacting proteins. The individual temporal profiles of selected Shc1-interacting proteins are shown (see Fig3b). Y-axis: relative abundance; X-axis: time after EGF addition (minutes). Results are representative of three biological replicates. Error bars are s.d. from all transitions for each protein from all technical repeats.



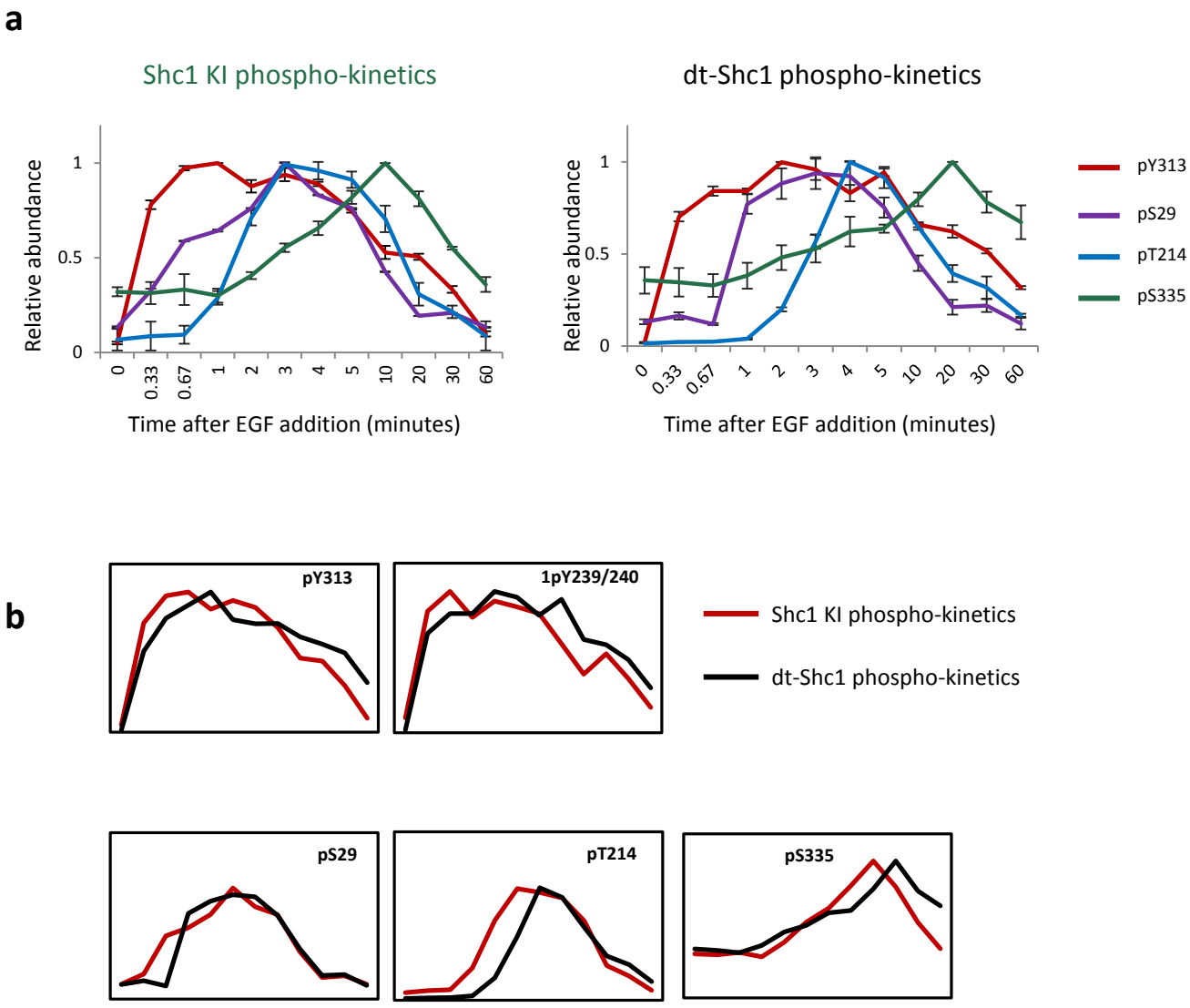
Supplementary Figure 12. Dynamic profiles of Cluster 1b Shc1-interacting proteins. The individual temporal profiles of Shc1-interacting proteins from Cluster 1b are shown (see Fig3b). Y-axis: relative abundance; X-axis: time after EGF addition (minutes). Results are representative of three biological replicates. Error bars are s.d. from all transitions for each protein from all technical repeats.



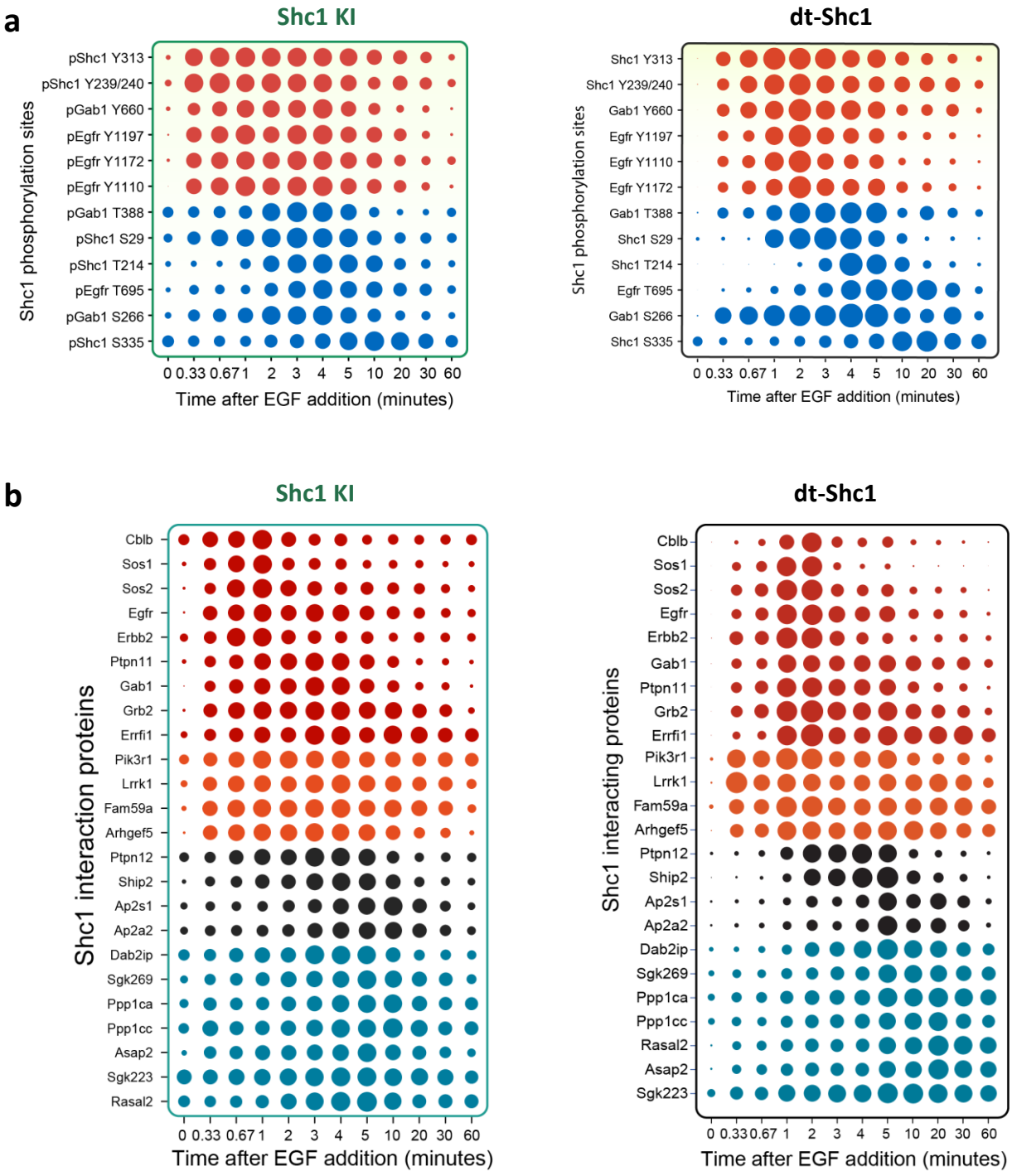
Supplementary Figure 13. Dynamic profiles of Cluster 2 Shc1-interacting proteins. The individual temporal profiles of Shc1-interacting proteins from Cluster 2 are shown (see Fig3b). Y-axis: relative abundance; X-axis: time after EGF addition (minutes). Results are representative of three biological replicates. Error bars are s.d. from all transitions for each protein from all technical repeats.



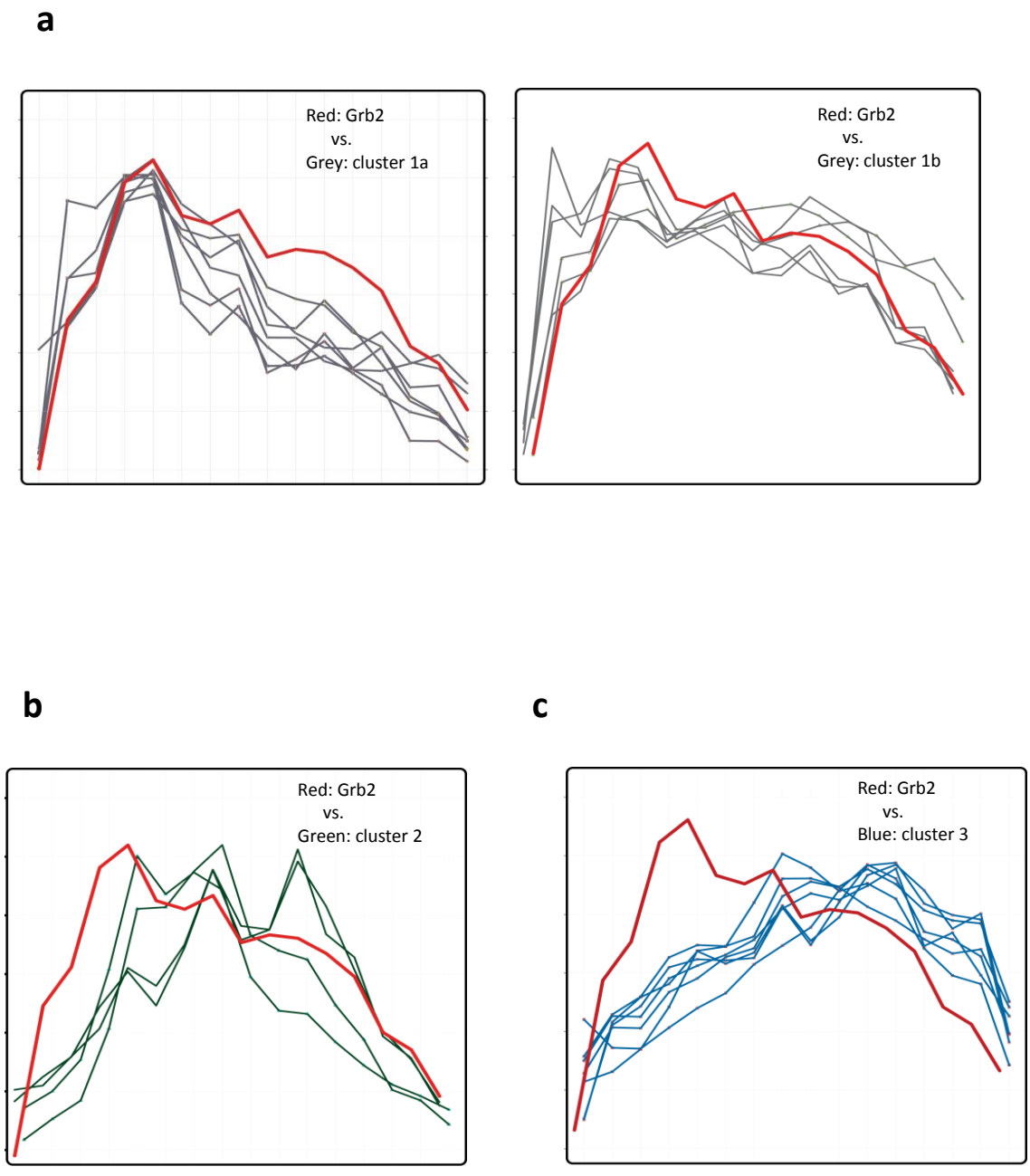
Supplementary Figure 14. Dynamic profiles of Shcbp1 and Cluster 3 Shc1 interacting proteins. The individual temporal profiles of Shc1-interacting proteins are shown (see Fig3b). Y-axis: relative abundance; X-axis: time after EGF addition (minutes). Results are representative of three biological replicates. Error bars are s.d. from all transitions for each protein from all technical repeats.



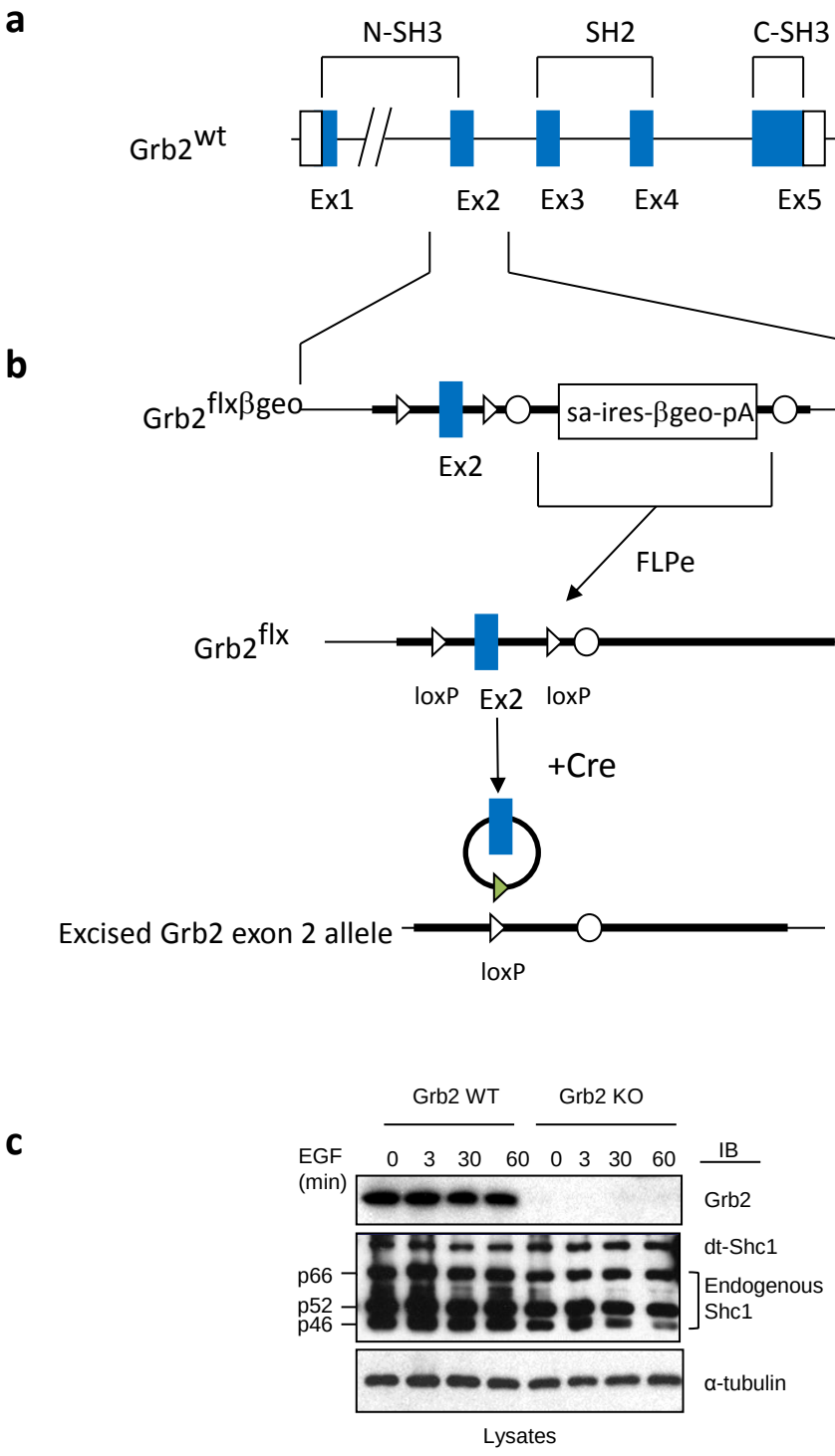
Supplementary Figure 15. Comparison of EGF-induced phosphorylation kinetics of endogenously expressed Shc1 vs. ectopically expressed dt-Shc1. a, Primary MEFs prepared from FLAG-tagged Shc1 knock-in (Shc KI) mice were stimulated with EGF for the indicated times. Phosphorylation kinetics of Shc1 KI were quantified by sMRM and compared with those from dt-Shc1 purified from stably expressing Rat-2 cells. Error bars are s.d. from all transitions for each protein from all technical repeats. Results are representative of three biological replicates. **b,** Overlay of phospho-kinetic curves of individual Shc1 sites.



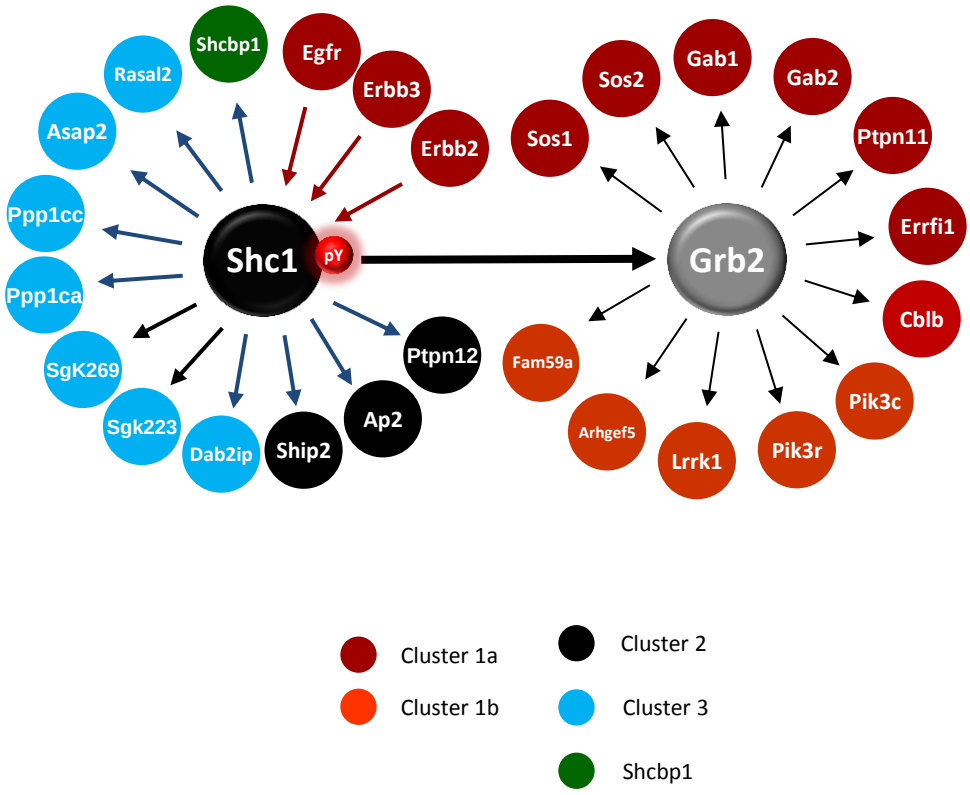
Supplementary Figure 16. Comparison of the temporal profiles of EGF-induced phosphorylation of the endogenous Shc1 complex as compared with the dt-Shc1 complex. a, Primary MEFs prepared from FLAG-Shc1 knock-in (Shc1 KI) mice were stimulated with EGF for the indicated times. Dynamic phosphorylation of the Shc1 complex purified from Shc1 KI cells was quantified by sMRM and compared with that from dt-Shc1-expressing cells. **b,** Temporal profiles of Shc1-associated proteins were compared from FLAG-Shc1 KI MEFs and dt-Shc1 Rat-2 cells stimulated with EGF.



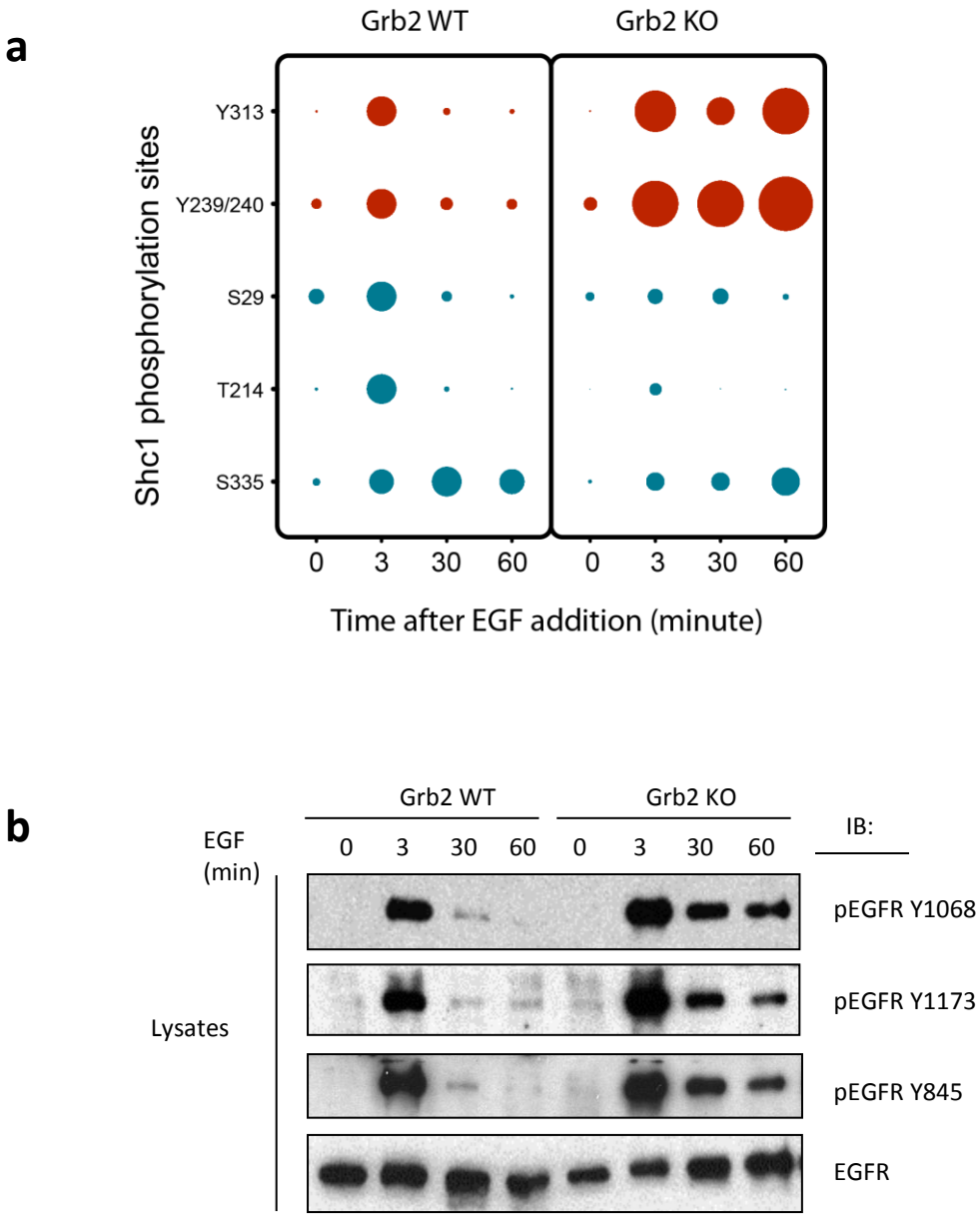
Supplementary Figure 17. Temporal profile comparisons. a, Shc1-associated Grb2 vs. Cluster 1a and 1b proteins. **b,** Shc1-associated Grb2 vs. Cluster 2 proteins. **c,** Shc1-associated Grb2 vs. cluster 3 proteins.



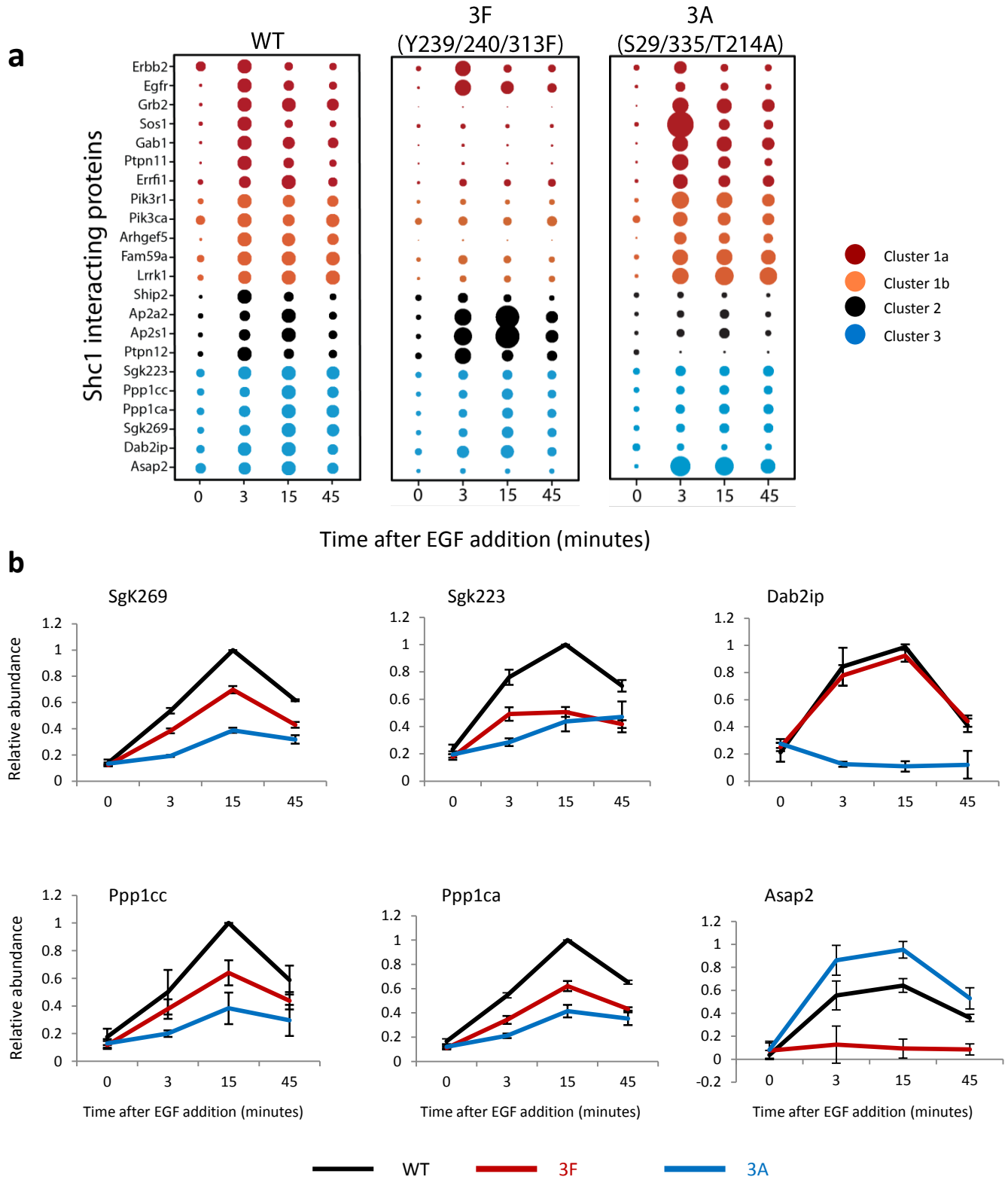
Supplementary Figure 18. Targeting of the mouse Grb2 gene with a conditional floxed allele. a, Diagrammatic representations of the wild-type mouse Grb2 gene. **b,** Cre-mediated deletion of exon 2 introduces a frameshift mutation. **c,** Grb2 levels after 4-OH-tamoxifen-induced excision in MEFs homozygous for the Grb2flox/flox allele and ectopically expressing dt-Shc1 (WT – wild-type MEFs, KO – Grb2-excised MEFs).



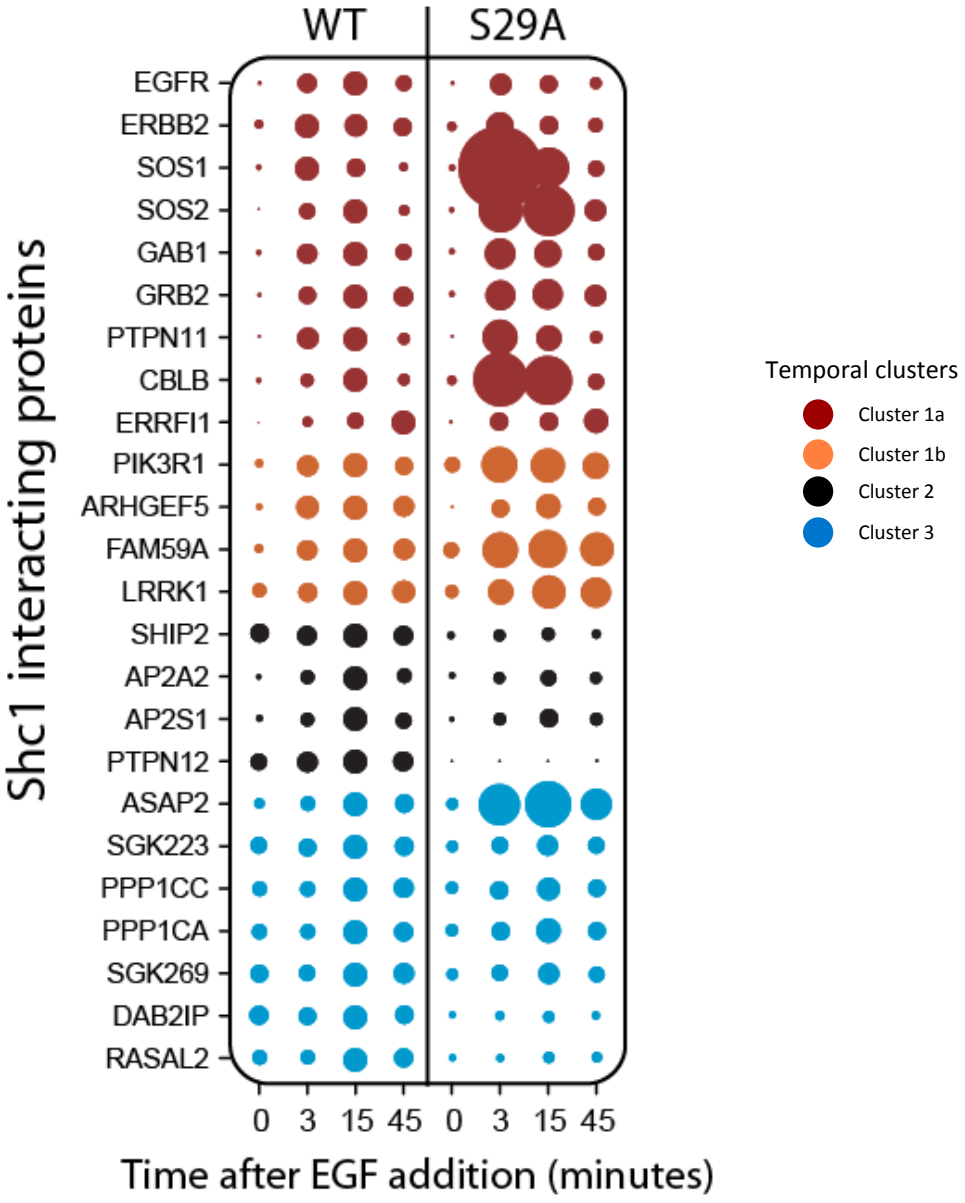
Supplementary Figure 19. EGF-induced Shc1-mediated signaling network consists of Grb2-dependent and -independent sub-networks. Experiments were performed as described in **Fig. 4b.** WT or Grb2 KO cells were stimulated with EGF for 5 minutes. Shc1 complexes were analyzed by sMRM. A summary view of the data is shown in which proteins are separated based on their requirement for or independence of Grb2 for Shc1 binding.



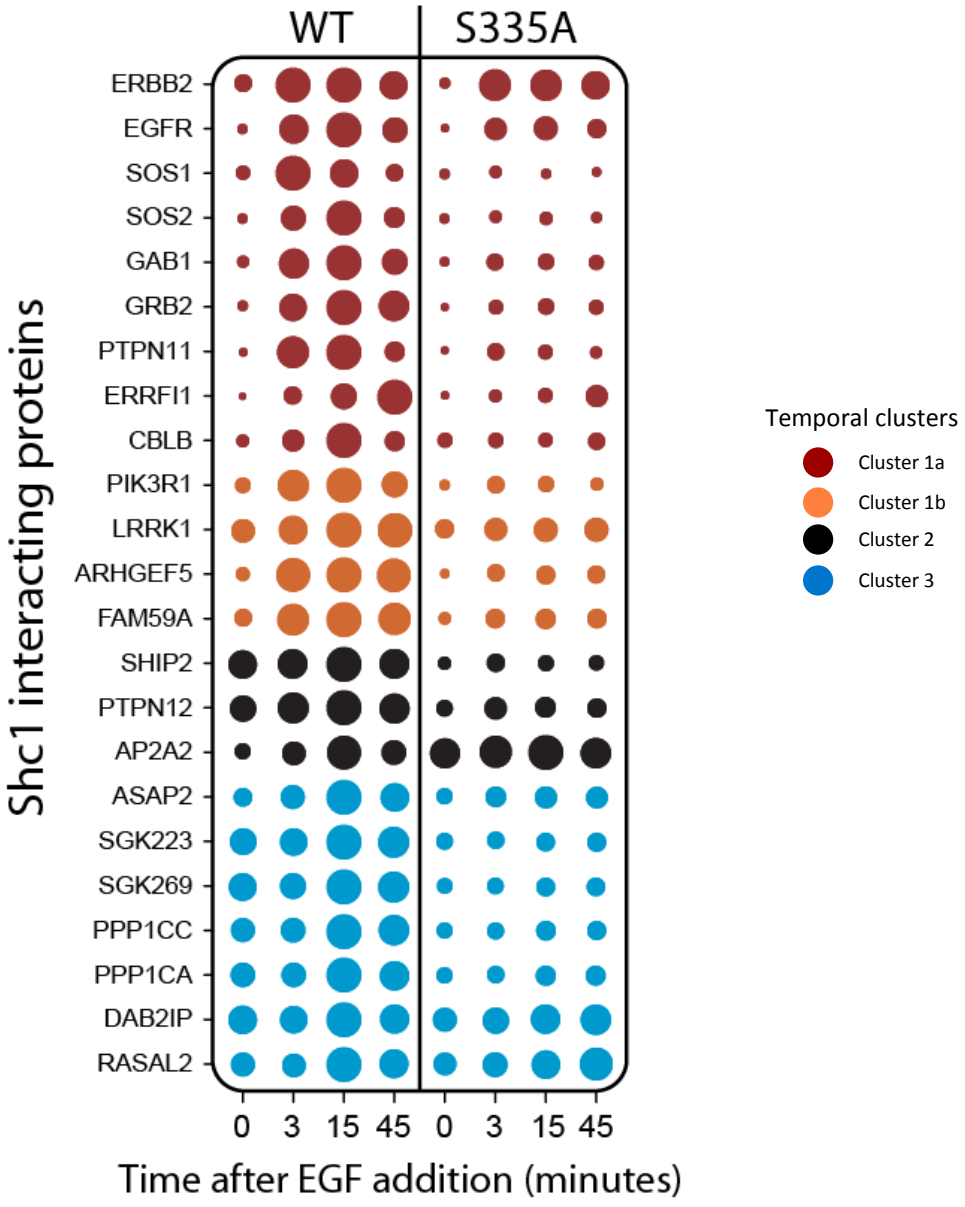
Supplementary Figure 20. Increased tyrosine phosphorylation on Shc1 and EGFR in Grb2-deficient cells. a, Grb2-deficient cells expressing dt-Shc1 were stimulated with EGF for the indicated times. The phosphorylation status of dt-Shc1 was analyzed by sMRM. **b,** Increased and sustained phosphorylation on various EGFR tyrosine sites in Grb2-deficient MEFs was analyzed by immunoblotting using phospho-specific antibodies.



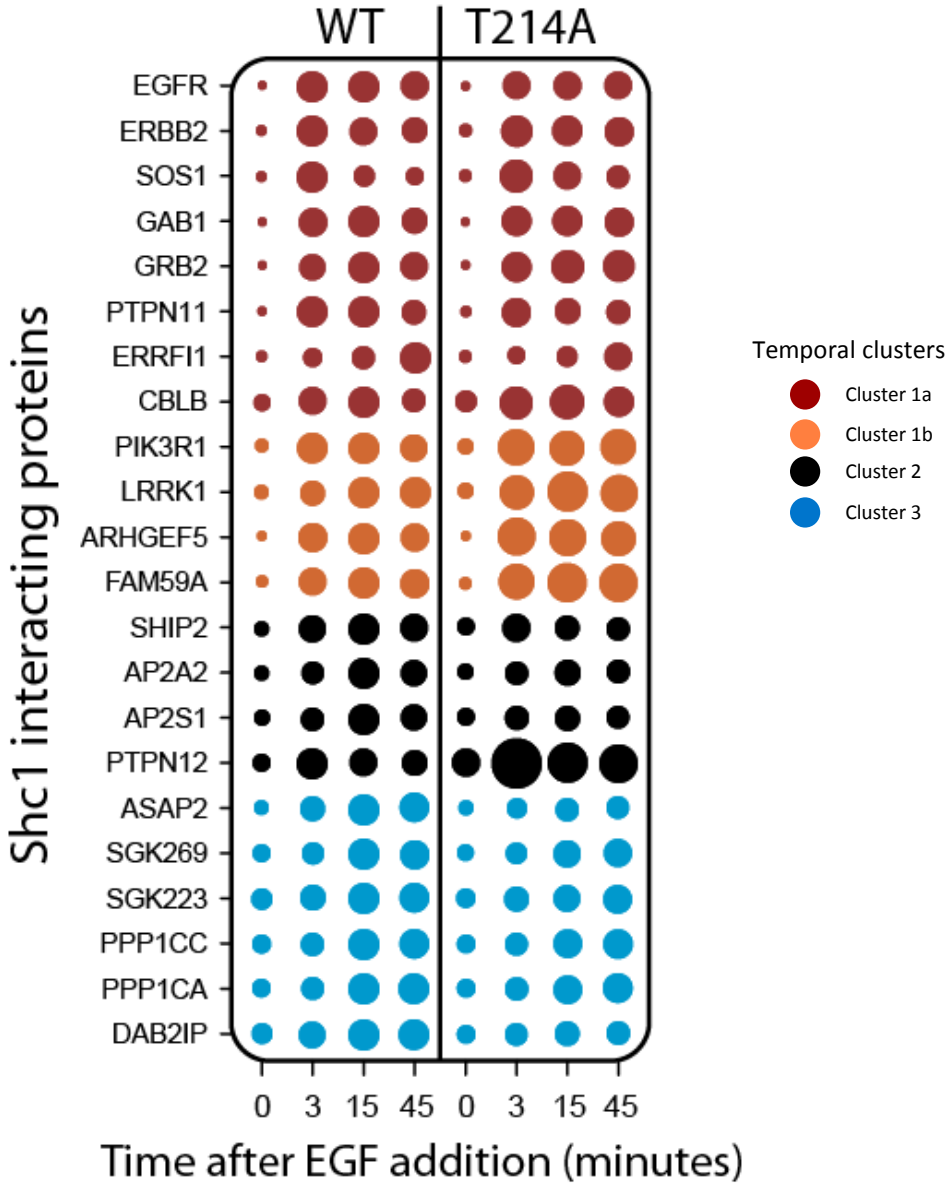
Supplementary Figure 21. Grb2-independent, serine/threonine-dependent Shc1 protein interactions. a, sMRM analysis of Shc1-interacting proteins in Shc1-deficient fibroblasts stably expressing wild-type dt-Shc1 (WT) or its mutants**. b,** Cluster 3 proteins were also plotted with dynamic curves for ease of comparison. 3F: Y239/240/313F; 3A: S29/335/T214A. Error bars are s.d. from all transitions for each protein from all technical repeats. Results are representative of two biological replicates.



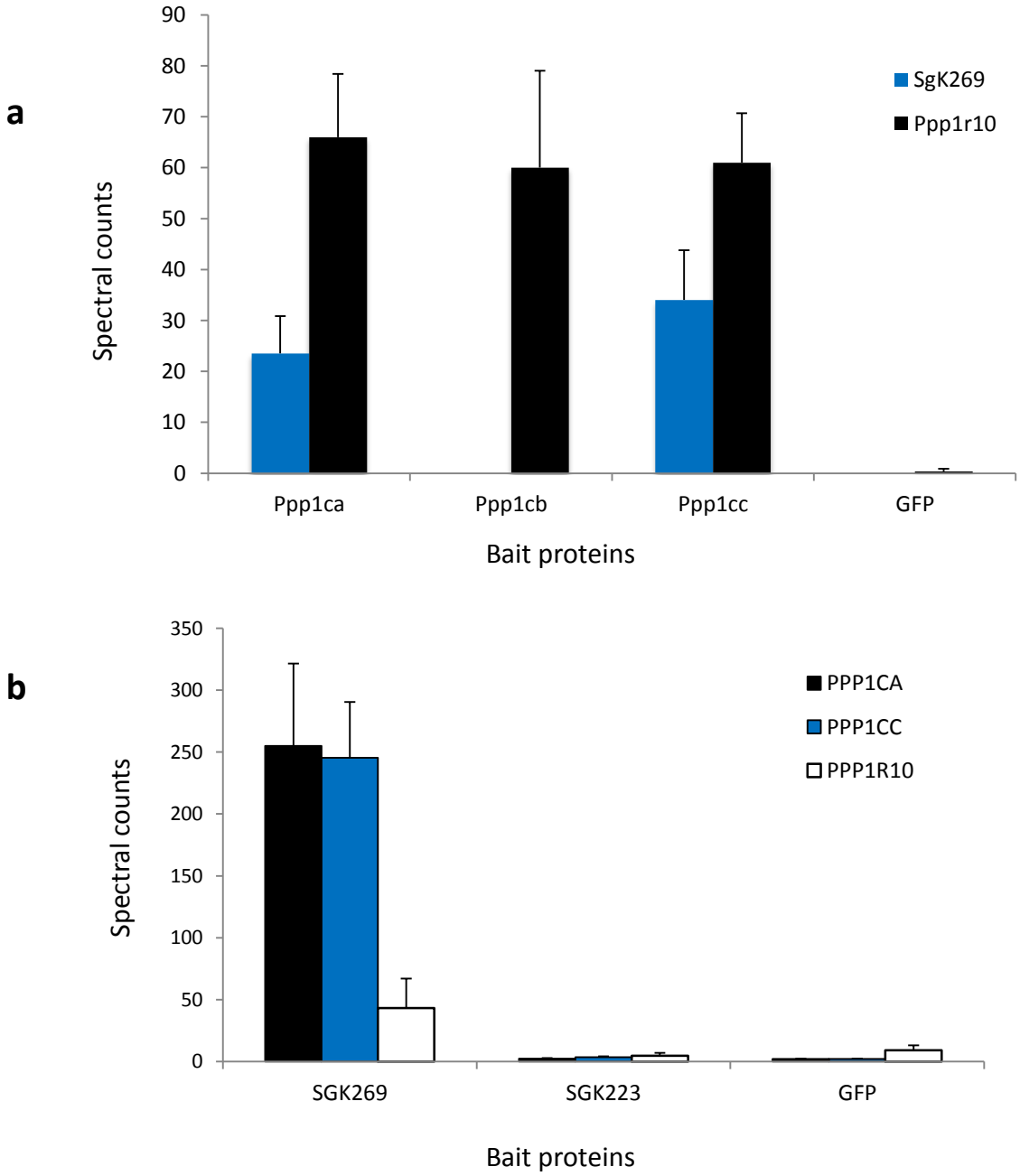
Supplementary Figure 22. Effect of alanine substitution of Shc1 Ser29 on EGF-induced Shc1 signaling complex assembly. sMRM analysis of Shc1-interacting proteins in Shc1-deficient fibroblasts stably expressing wild-type dt-Shc1 (WT) or S29A mutant. Note: For each protein, the highest value for association with WT Shc1 is set as 100%.



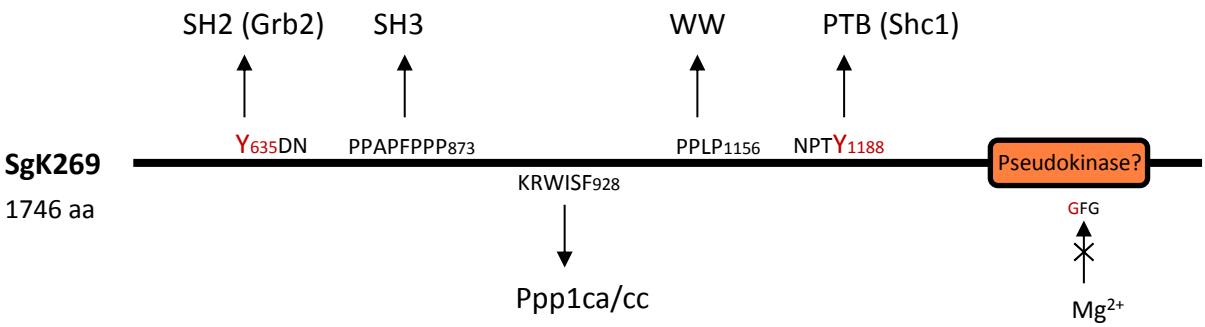
Supplementary Figure 23. Effect of alanine substitution of Shc1 Ser335 on EGF-induced Shc1 signaling complex assembly. sMRM analysis of Shc1-interacting proteins in Shc1-deficient fibroblasts stably expressing wild-type dt-Shc1 (WT) or S335A mutant. Note: For each protein, the highest value for association with WT Shc1 is set as 100%.



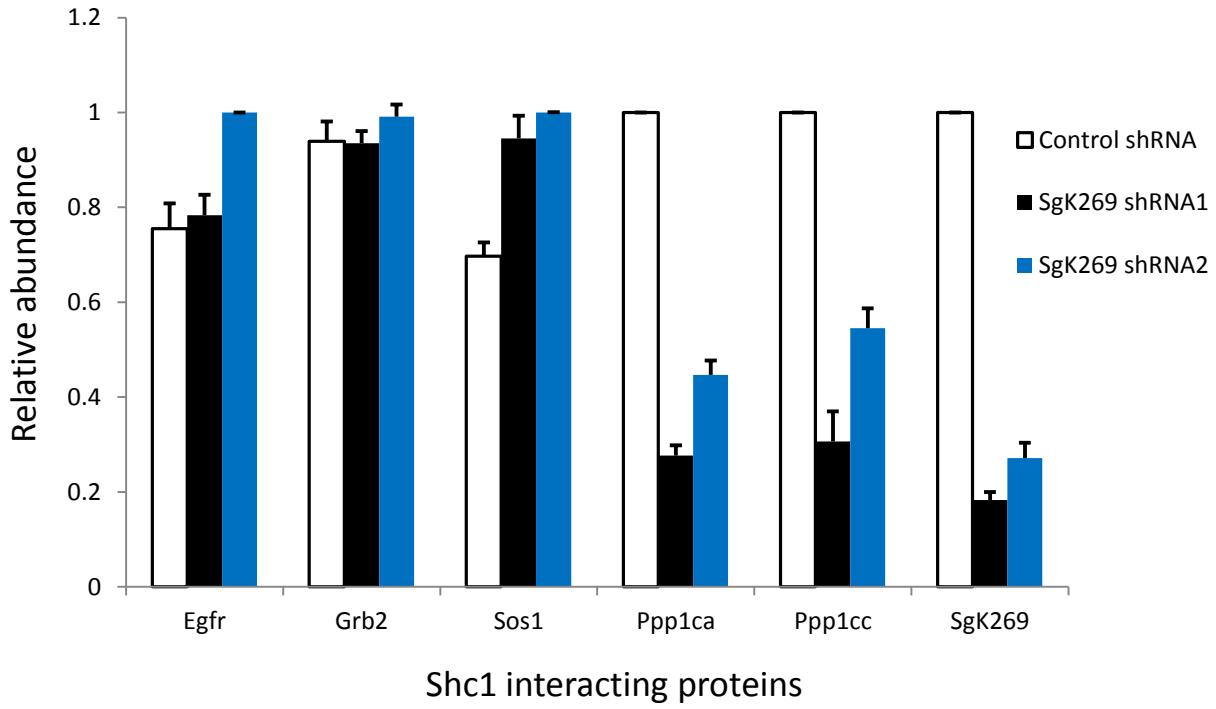
Supplementary Figure 24. Effect of alanine substitution of Shc1 Thr214 on EGF-induced Shc1 signaling complex assembly. sMRM analysis of Shc1-interacting proteins in Shc1-deficient fibroblasts stably expressing wild-type dt-Shc1 (WT) or T214A mutant. Note: For each protein, the highest value for association with WT Shc1 is set as 100%.



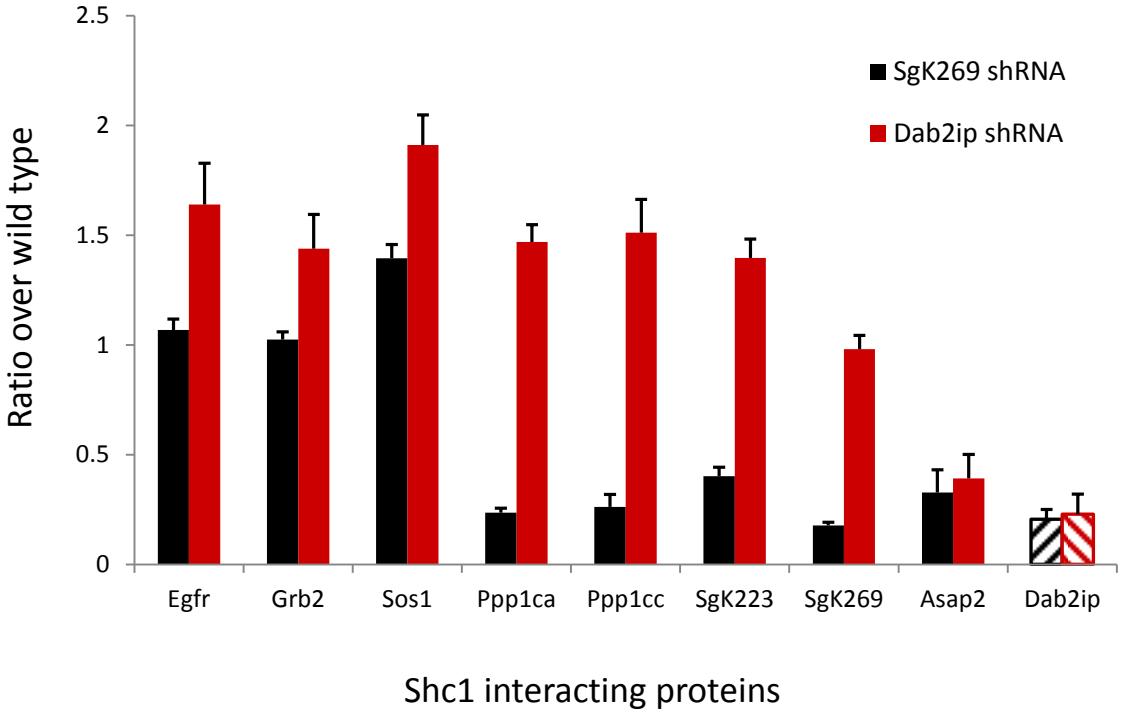
Supplementary Figure 25. SgK269 specifically associates with Ppp1ca and Ppp1cc. a, Normalized spectral counts (across all replicates and conditions) for the interactions of SgK269 and Ppp1r10 with each of the three catalytic subunits of PP1 and with the negative control GFP. FLAG-tagged Ppp1ca, Ppp1cb, Ppp1cc and GFP were purified from stably-transfected HEK293 cells and then analyzed by LC/MS. SgK269 is highly enriched with Ppp1ca and Ppp1cc, but not detected as an interaction partner for Ppp1cb. By contrast, Ppp1r10 (also known as PNUTS) interacts with all three of the catalytic subunits (n≥13). **b,** SgK269, but not SgK223, associates with Ppp1ca and Ppp1cc. FLAG-tagged SgK269, SgK223 and GFP were overexpressed and purified from EGF-stimulated HEK293 cells, then analyzed by LC/MS. Normalized spectral counts of Ppp1ca, Ppp1cc and Ppp1r10 are shown. n=3. Error bars indicate s.d. from all transitions of each protein from all technical repeats.



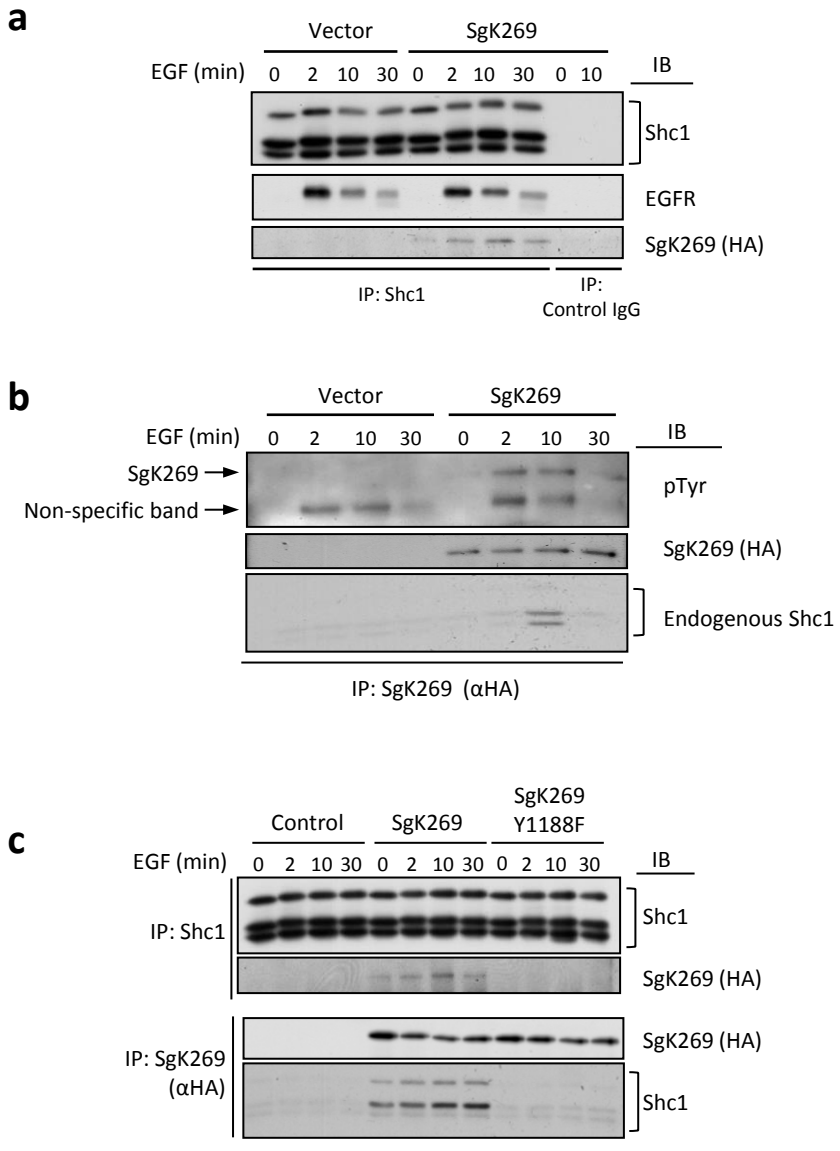
Supplementary Figure 26. SgK269 contains multiple motifs involved in protein-protein interactions. Potential motifs are predicted based on experimental data, literature searches and bioinformatics analyses. SgK269 lacks the conserved Asp residue within the conserved DFG motif, critical for binding Mg2+ ions in protein kinases. This is replace by a Gly residue (highlighted in red). Phosphorylated tyrosine sites are highlighted in red.



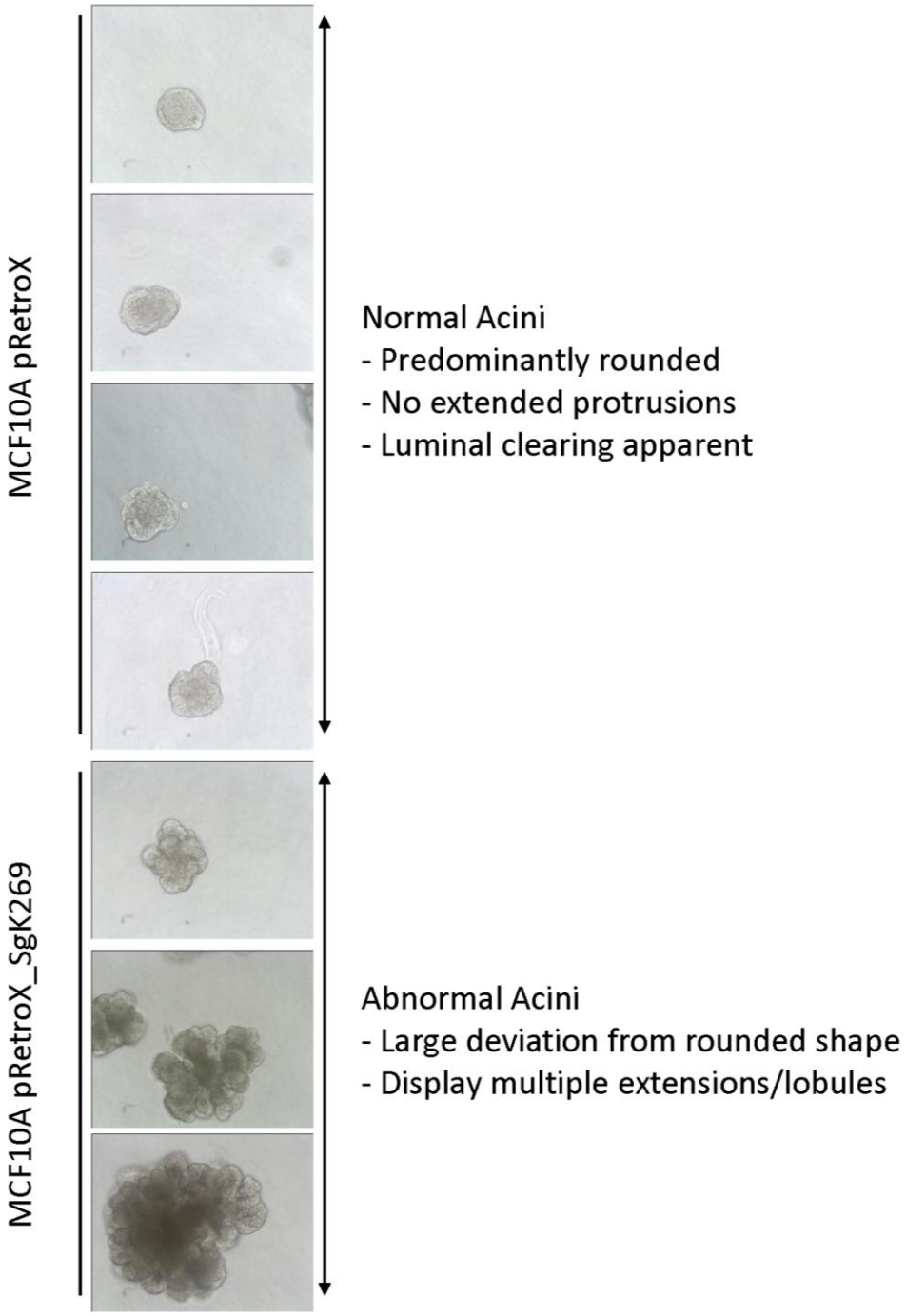
Supplementary Figure 27. Lentiviral-mediated knock-down of SgK269 effectively decreases EGF-dependent binding of Ppp1ca and Ppp1cc to Shc1 in HeLa cells. Cells infected with SgK269 shRNA1 (black bars), SgK269 shRNA2 (blue bars), or luciferase shRNA (open bars) were stimulated with EGF. Endogenous Shc1 complexes were purified by immunoprecipitation using an anti-Shc antibody. The relative abundances of Shc1-interacting proteins were quantified by sMRM. Error bars are s.d. from all transitions for each protein from all technical repeats. Results are representative of three biological replicates.



Supplementary Figure 28. Lentiviral-mediated knockdown of Dab2ip protein expression does not affect EGF-dependent association of SgK269-Ppp1ca/cc complex to the Shc1 complex. HeLa cells infected with lentiviruses expressing shRNAs for luciferase, SgK269, or Dab2ip were stimulated with EGF. The Shc1 signaling complex was affinity purified with anti-Shc antibody. The indicated Shc1-interacting proteins were quantified by sMRM. Striped bars identify proteins that are the targets of shRNA knockdown. Ratio over wild type=Relative abundance of Shc1 binding partners in SgK269 or Dab2IP knockdown cells/Relative abundance of Shc1 binding partners in control knockdown cells. Error bars are s.d. from all transitions for each protein from all technical repeats. Results are representative of two biological replicates.



Supplementary Figure 29. SgK269 is tyrosine phosphorylated upon EGF stimulation. a, Shc1 interacts with SgK269 with delayed kinetics following EGF stimulation. EGF-stimulated MCF10A cells stably expressing myc-SgK269-HA (SgK269) or empty vector were immunoprecipitated with anti-Shc1 antibodies and immunoblotted as indicated. **b,** MCF10A cells stably expressing myc-SgK269-HA (SgK269) or empty vector were serum-starved and stimulated with EGF for the indicated times. HA-tagged SgK269 was affinity purified. Tyrosine phosphorylation of SgK269 was examined using pTyr-specific antibodies (top panel), and associated Shc1 identified by blotting with anti-Shc antibodies (lower panel). **c**, EGF-induced association of the SgK269 Y1188F mutant with Shc1 was examined using immunoprecipitation and immunoblotting.



Supplementary Figure 30. Morphology of SgK269-induced MCF10A acini. MCF10A cells stably expressing empty vector (pRetroX) or SgK269 were grown in Matrigel for 12 days and photographed. Overexpression of WT SgK269 within this model system generates acini with a two-fold increase in diameter over those of control acini. Furthermore, these enlarged acini present with a multi-lobular morphology and non-cleared lumens, distinct from those of the rounded, hollow control acini. Both of these phenotypes induced by over-expression of WT SgK269 are rescued by mutation of Y1188 to phenylalanine. This tyrosine residue sits within a canonical NPXY motif, responsible for the interaction between SgK269 and Shc1.