Supplementary Information

Ms. Title: "Identification of a novel interaction between Annexin A2 and Keratin 17: Evidence for reciprocal regulation"

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Supplementary Experimental Procedures

In-gel digestion— Proteins were silver stained and bands of interest along with a control were excised, reduced, alkylated and in-gel digested following the procedure outlined in (1). Peptides were extracted by 15 min incubation in 5% (v/v) formic acid followed by the addition of an equal volume of 100% ACN and 15 min incubation, this step was repeated and the extracts were combined.

Mass spectrometry analysis— Proteins were identified using an Orbitrap LTQ tandem mass spectrometer (Thermo Fisher, Waltham, MA) coupled with an Agilent 1200 nanoLC (Agilent Technologies, Santa Clara, CA). Peptides eluted from the gels slices were separated on a C18 RP-HPLC column (75 μm x 10 cm self-packed with 5 μm, 200 Å Magic C18; Michrom BioResources, Auburn, CA) at a flow rate of 300 nL / min with mobile phase A, 0.1% v/v formic acid in water, and mobile phase B, 90% acetonitrile, 0.1 % formic acid in water. Peptides were eluted with a linear gradient from 10% B to 45% B over 15 minutes and then up to 65% B in 5 min. Each MS1 scan followed by collision induced dissociation (CID) of the five most abundant precursor ions with dynamic exclusion for 30 seconds. Only MS1 signals exceeding 12,000 counts triggered the MS2 scans. For MS1, $2x10^5$ ions were accumulated in the Orbitrap over a maximum time of 500 ms and scanned at a resolution of 60,000 FWHM (at 400 m/z). MS2 spectra were acquired in normal scan mode in the LTQ, a target setting of 10^4 ions and accumulation time of 30 ms. The normalized collision energy was set to 35%, and one microscan was acquired for each spectrum.

MS Data analysis—MS data was searched against the International Protein Index mouse (v3.62, 56733 entries) or human (v3.62, 83947 entries) primary sequence database (2) using Sorcerer 2™-SEQUEST® (version v.27, rev. 11) (Sage-N Research, Milpitas, CA) with post search analysis performed using Scaffold 2 (Proteome Software Inc. Portland, OR) implementing the PeptideProphet [3] and ProteinProphet [4] algorithms. All raw data peak extraction was performed using the Sorcerer 2™-SEQUEST® default settings. Search parameters included semi-enzyme digest with trypsin (after Arg or Lys) with up to 2 missed cleavages. SEQUEST® was searched with a parent ion tolerance of 0.15 Da and a fragment ion mass tolerance of 1.00 Da with carbamidomethylation (C) specified as a differential modification. Peptide identifications were accepted if they had an Xcorr score greater than 2.0, a probability greater than 95.0% as specified by the PeptideProphet algorithm (3), and met with visual validation. Protein identifications were accepted if they could be established at greater than 99.0% probability as assigned by the ProteinProphet algorithm (4) and contained at least 2 identified peptides. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Entries not present in the mouse database were

assigned the human sequence. Identifications determined from the control gel slice were considered to be background and subtracted from the analysis.

Reference List for Supplementary Experimental Procedures

1. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) *Anal. Chem.* **68**, 850-858

2. Kersey, P. J., Duarte, J., Williams, A., Karavidopoulou, Y., Birney, E., and Apweiler, R. (2004) *Proteomics* **4**, 1985-1988

3. Keller, A., Purvine, S., Nesvizhskii, A. I., Stolyar, S., Goodlett, D. R., and Kolker, E. (2002) *OMICS* **6**, 207-212

4. Nesvizhskii, A. I., Keller, A., Kolker, E., and Aebersold, R. (2003) *Anal. Chem.* **75**, 4646-4658

Supplemental Figure Legends

Supplemental Table T1. LC/MS/MS-based identification of AnxA2 protein in K17 immunoprecipitates.

Supplemental Figure S1. EGFR alters K17 solubility.
Growth factor-deprived A431 cells were treated with (B) or without (A) 100 ng/ml EGF for 1 h then immunostained with an anti-K17 antibody. Each bar represents 20 μm.

Supplemental Figure S2. Identification of candidate binding partners for K17 by LC/MS/MS.
A. Immunoprecipitation was performed from 308 mouse keratinocyte cell lysate along with an anti-K17 antibody (K17) or pre-immune serum (PIS) as a control. Immunoprecipitates were electrophoresed (SDS-PAGE) followed by silver staining. Protein bands of interest were excised and identified by LC/MS/MS. Bracket indicates number of unique peptides observed. B. Annotated spectra for four peptides assigned to Annexin A2 (QDIAFAYQR; TPAQYDASELK; TNQELQEINR; GVDEVTIVNILTNR).

Supplemental Figure S3. AnxA2 and K17 localization.
mCherry-AnxA2 or mCherry and GFP-K17 or GFP were expressed in A431 cells and fluorescence images recorded using confocal microscopy. Each bar represents 20 μm. Weighted colocalization coefficients were determined using the LSM Image Examiner software. Weighted colocalization coefficients were normalized to the values from mCherry-AnxA2 and GFP-K17 co-expression image, and relative weighted colocalization coefficients are shown. For each indicated protein, the value corresponds to the degree that its channel colocalizes with another channel. The relative coefficient for untagged mCherry and GFP controls that colocalize with GFP-K17 and mCherry-AnxA2 (respectively) were lower than mCherry-AnxA2 that colocalizes with GFP-K17 and GFP-K17 that colocalizes with mCherry-AnxA2.

Supplemental Figure S4. AnxA2 interacts with K5.
A. Immunoprecipitation was performed with anti-K5 antibody (K5) or rabbit IgG (IgG) as control in A431 cells, and immunoblotting was performed with antibodies against the indicated proteins. B. mCherry-tagged AnxA2 and GFP-K5 were expressed (via transient transfection) in 293T cells. Immunoprecipitation was performed with anti-K5 antibody (K5) or rabbit IgG (IgG) as a control, and immunoblotting performed with antibodies against the indicated proteins.

Supplemental Figure S5. EGF induces change in AnxA2 localization.
A. Growth factor-deprived A431 cells were treated with (+EGF) or without (-EGF) 100 ng/ml EGF for 30 min followed by immunostaining using anti-K17 and anti-AnxA2 antibodies. Each bar represents 20 μm. B. Weighted colocalization coefficients were determined using the LSM Image Examiner software. Weighted colocalization coefficients were normalized to the values from "–EGF" (control) treatments. Relative weighted colocalization coefficients of AnxA2 and K17 are shown. (*) indicates p value < 0.04 and (**) indicates p<0.03 for "+EGF" compared to "–EGF" treatments.

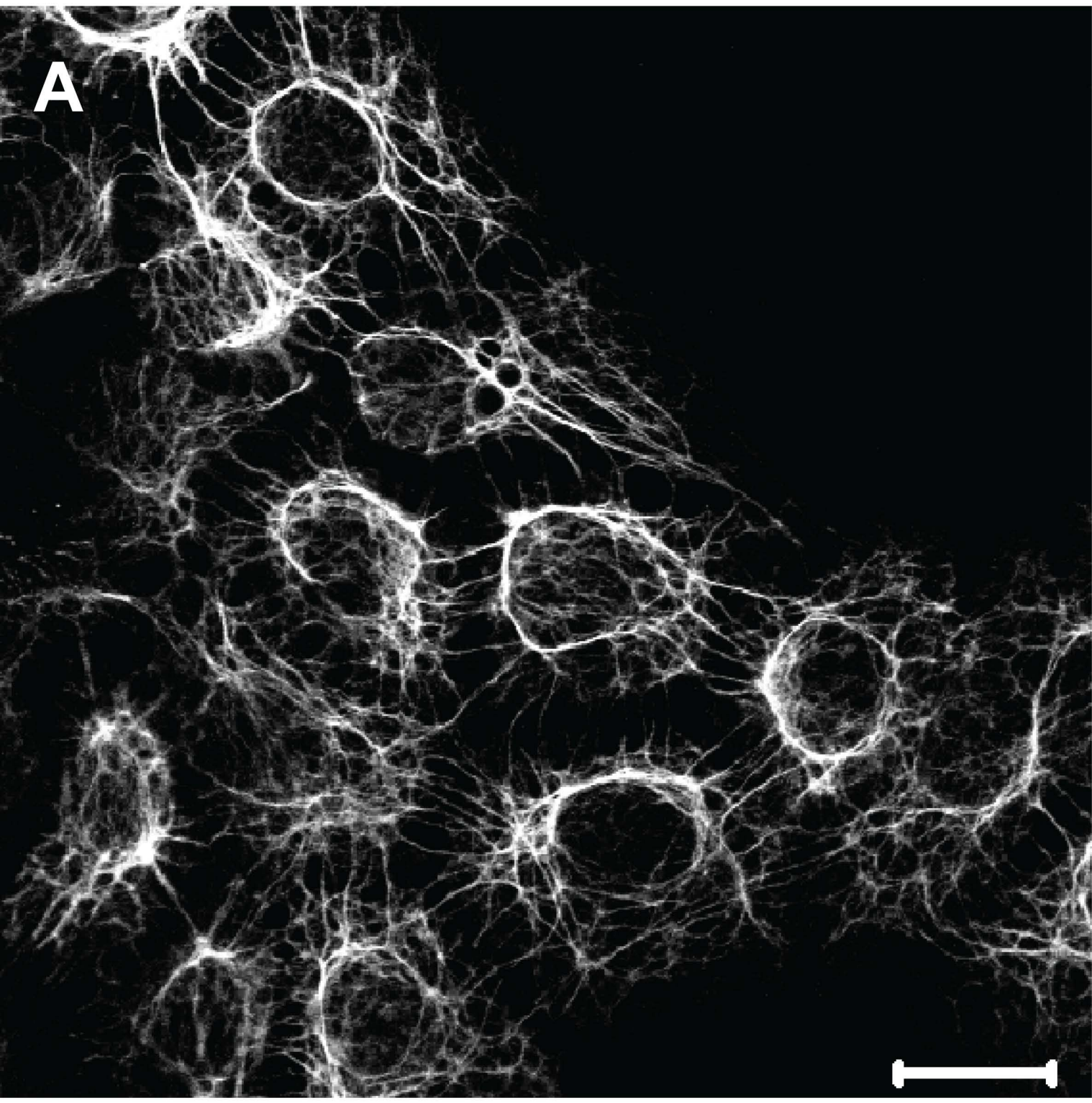
Supplemental Figure S6. EGF-induced K17 solubility change in AnxA2 KD cells.
A. A431 cells stably expressing vector control or AnxA2 shRNA were growth-factor deprived, then treated with 100 ng/ml EGF for indicated time period. Whole cell lysates were prepared in triton lysis buffer. Immunoblotting was performed with antibodies against the indicated proteins, and signal intensities were quantified using AlphaView SA. B. Quantitation of panel A. Relative K17 levels were normalized to those observed in the absence of EGF stimulation (0 min time point).

Supplemental Figure S7. EGFR activity is not affected by K17 expression status.
A. A431 cells with stable knockdown of K17 or vector control were growth factor-deprived and then stimulated with the indicated concentration of EGF for 30 min. B. Skin keratinocytes were obtained from $K17^{+/-}$ or $K17^{-/-}$ newborn mice and seeded for primary culture. For both A and B, whole cell lysates were prepared in triton lysis buffer, and immunoblotting was performed with antibodies against the indicated proteins. Anti-phosphotyrosine (PY) 4G10 antibody was used to assess EGFR activity.

Supplemental Table T1

Band	Protein name	Species	Protein accession number	Uniprot accession number	Molecular weight (kDa)	Protein identification probability	Number of unique peptides	Percentage sequence coverage	Peptide sequence	Peptide probability	XCorr	deltaCn	Enzymatic termini	Observed Mass	Actual Mass	Charge	Delta AMU	Delta PPM	Modifications	Spectrum ID
1	KRT17: Keratin, type I cytoskeletal 17	mouse	IPI00230365	Q9QWL7	48.1	100.00%	8	17.10%	(R)VLDELTLAR(A)	95%	3.2514	0.426	2	515.3036	1,028.59	2	0.005856	5.688		band-1-A1.03449.03449.2.dta
									(R)LASYLDKVR(A)	95%	2.8095	0.1567	2	532.8132	1,063.61	2	0.009243	8.682		band-1-A1.02937.02937.2.dta
									(R)TIVEEVQDGK(V)	95%	2.6641	0.2069	2	559.2944	1,116.57	2	0.007723	6.91		band-1-A1.02857.02857.2.dta
									(R)LEQEIATYR(R)	95%	2.9096	0.2946	2	561.7966	1,121.58	2	0.00694	6.182		band-1-A1.02851.02851.2.dta
									(R)TKFETEQALR(L)	95%	3.0366	0.2366	2	611.8293	1,221.64	2	0.008669	7.09		band-1-A1.02680.02680.2.dta
									(R)ALEEANTELEVK(I)	95%	3.2728	0.3807	2	673.3504	1,344.69	2	0.008884	6.602		band-1-A1.03042.03042.2.dta
									(R)EVATNSELVQSGK(S)	95%	3.6851	0.4705	2	681.3563	1,360.70	2	0.01462	10.74		band-1-A1.02606.02606.2.dta
									(K)TRLEQEIATYR(R)	95%	4.2776	0.4264	2	690.373	1,378.73	2	0.01085	7.864		band-1-A1.03122.03122.2.dta
2	ANXA2: Annexin A2	mouse	IPI00468203,IPI00605518,IPI00	P07356	24.4	100.00%	4	20.10%	(R)QDIAFAYQR(R)	95%	2.2191	0.2736	2	556.2856	1,110.56	2	0.01074	9.667		band3-A3.03033.03033.2.dta
									(K)TPAQYDASELK(A)	95%	3.5313	0.3153	2	611.8078	1,221.60	2	0.01318	10.78		band3-A3.02667.02667.2.dta
									(R)TNQELQEINR(V)	95%	3.3224	0.2292	2	622.8204	1,243.63	2	0.01043	8.376		band3-A3.02617.02617.2.dta
									(K)GVDEVTIVNILTNR(S)	95%	2.3889	0.3427	2	771.9344	1,541.85	2	0.01287	8.344		band3-A3.04312.04312.2.dta

Supplemental Figure S1



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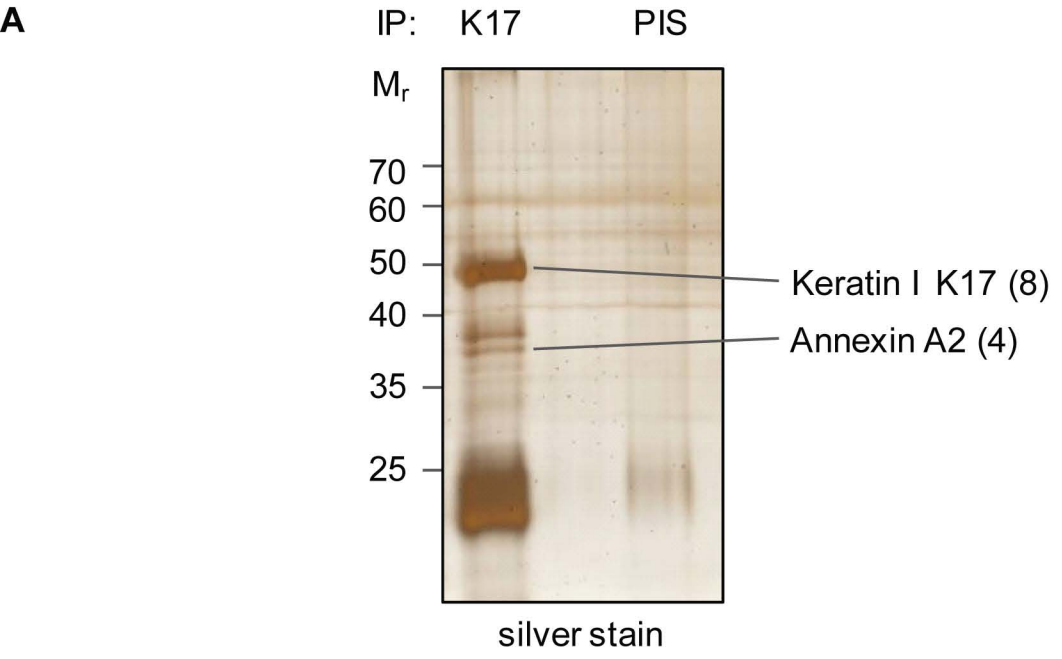
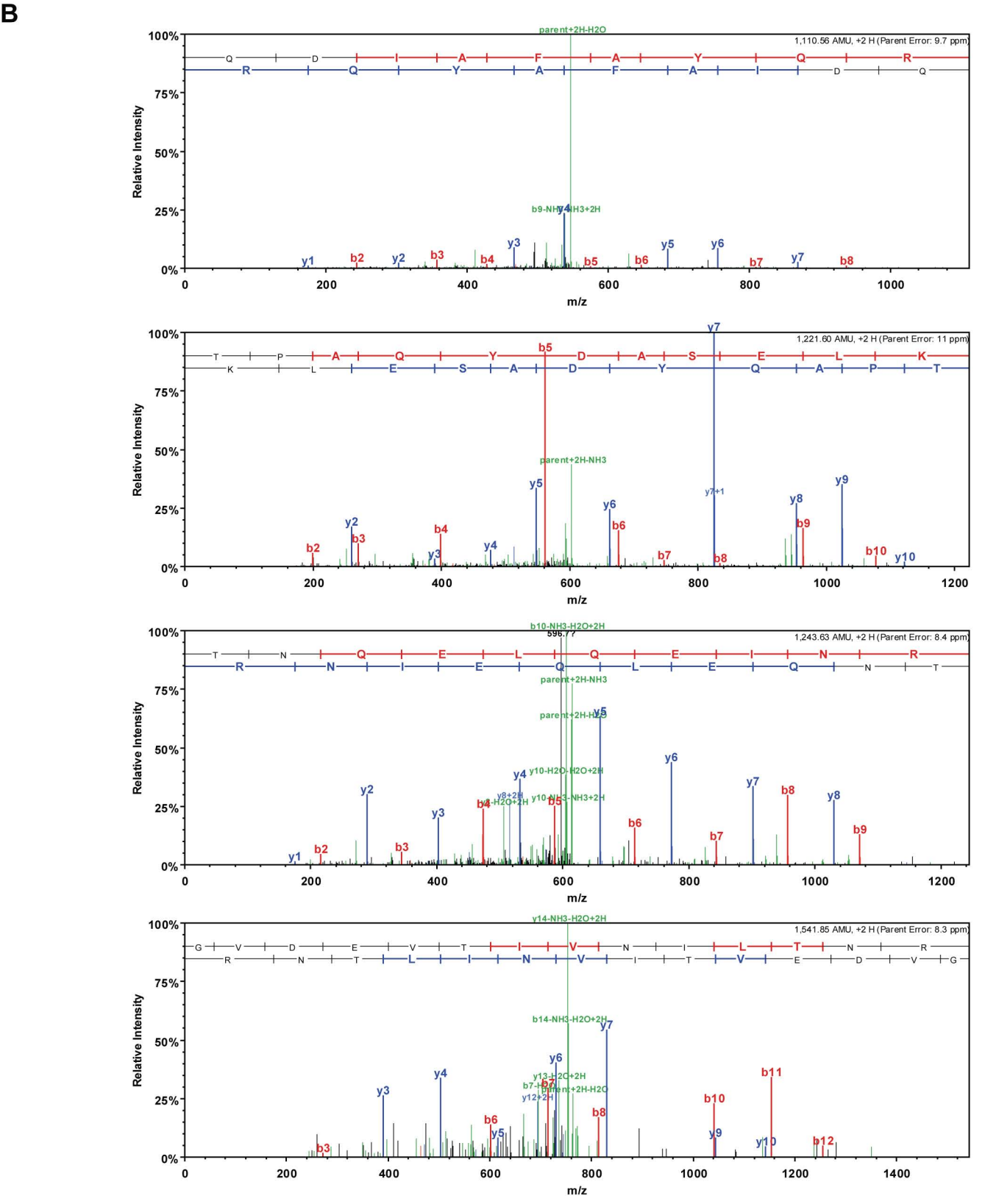
Supplemental Figure S2





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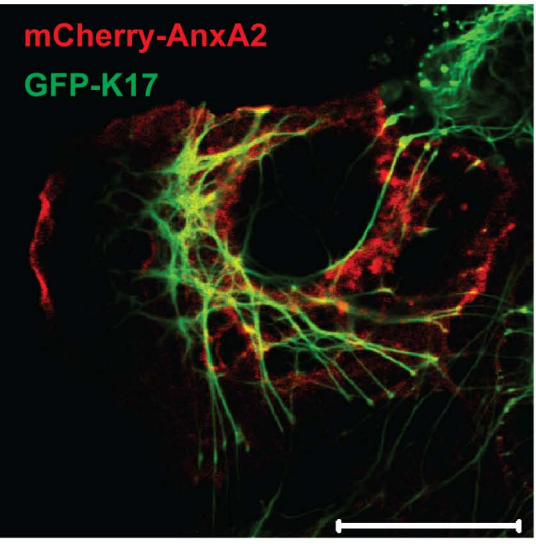
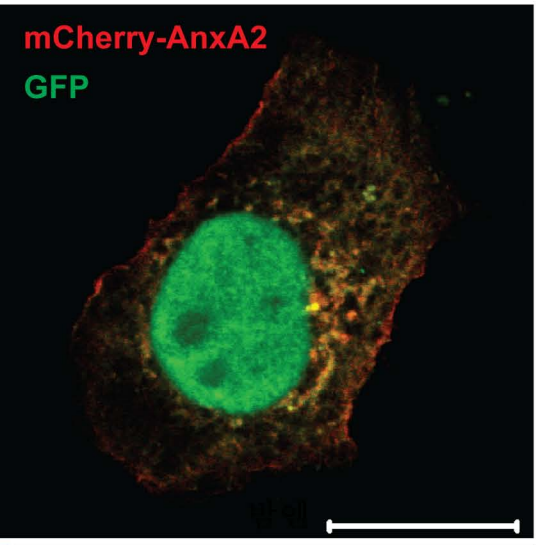
Supplemental Figure S3



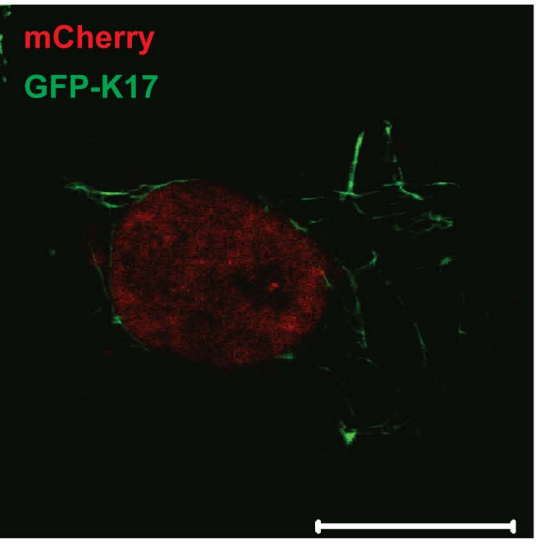
mCherry-AnxA2: 1.0

GFP-K17: 1.0



mCherry-AnxA2: 0.79

GFP: 0.15



mCherry: 0

GFP-K17: 0

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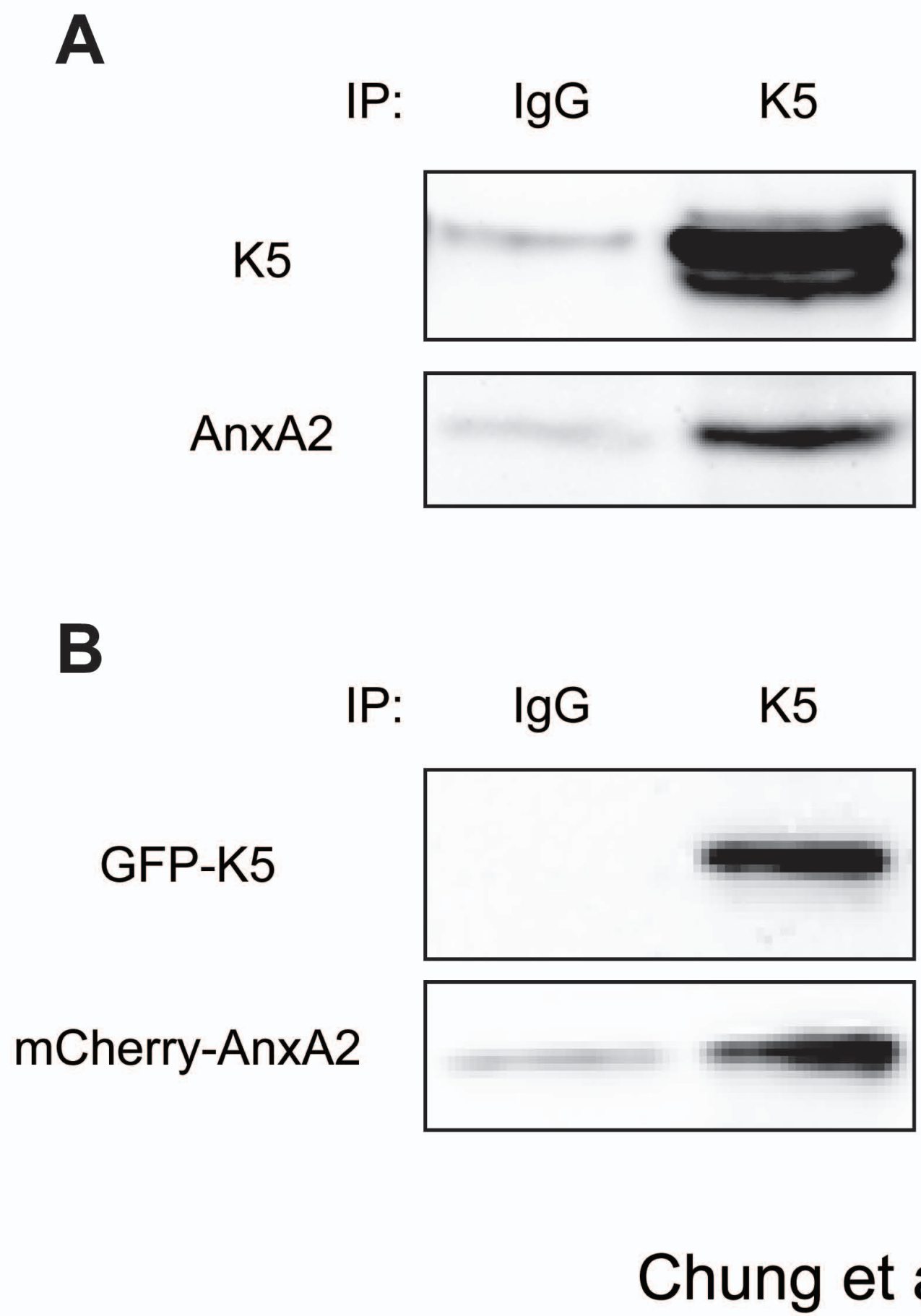
Supplemental Figure S4



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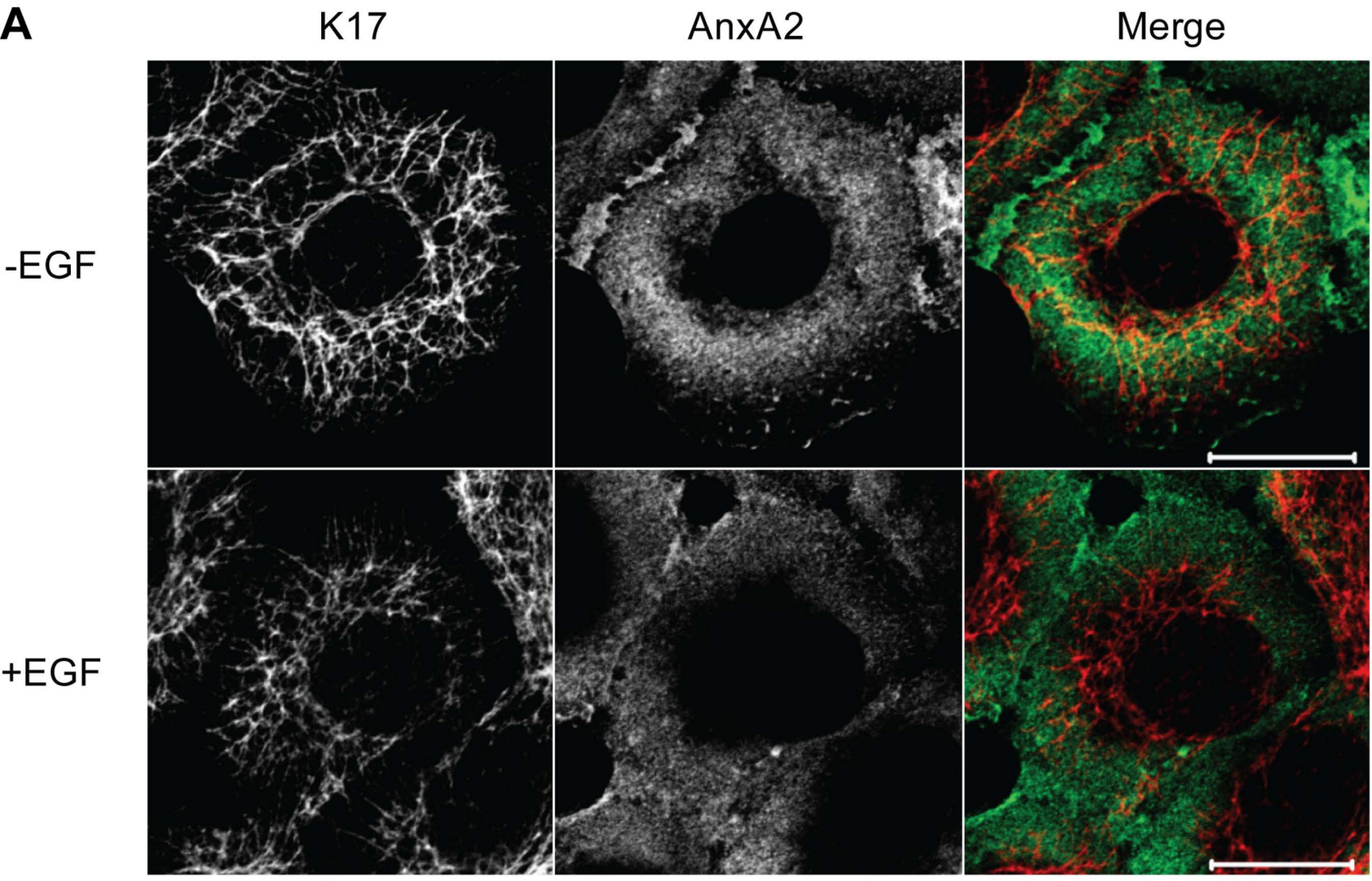
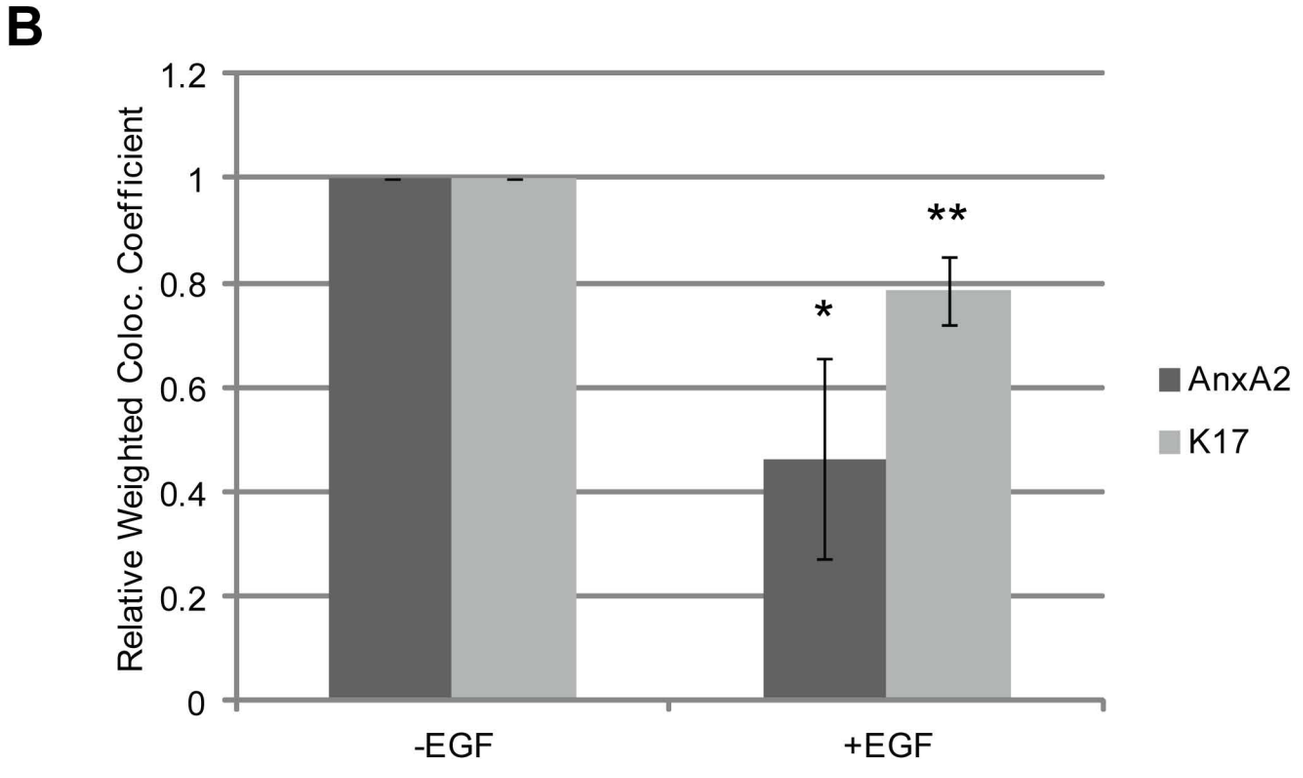
Supplemental Figure S5





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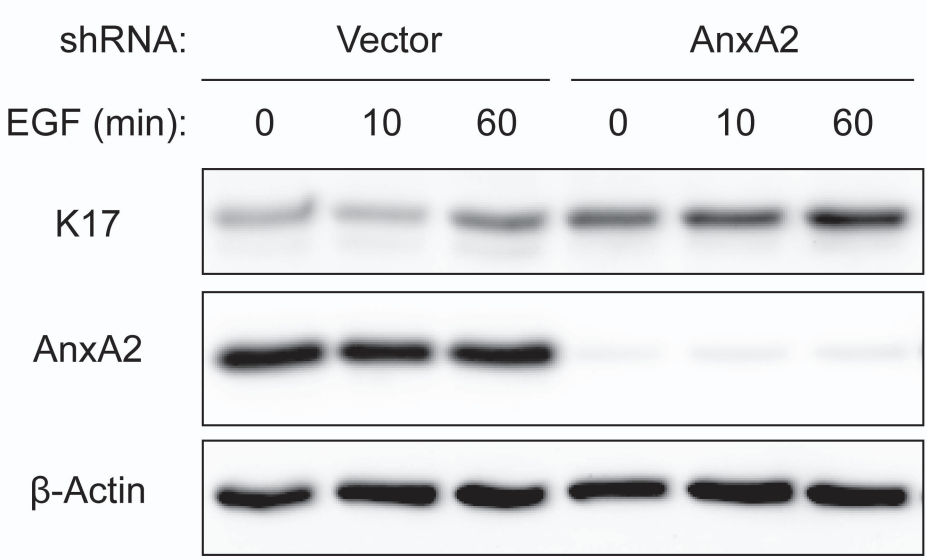
Supplemental Figure S6

A



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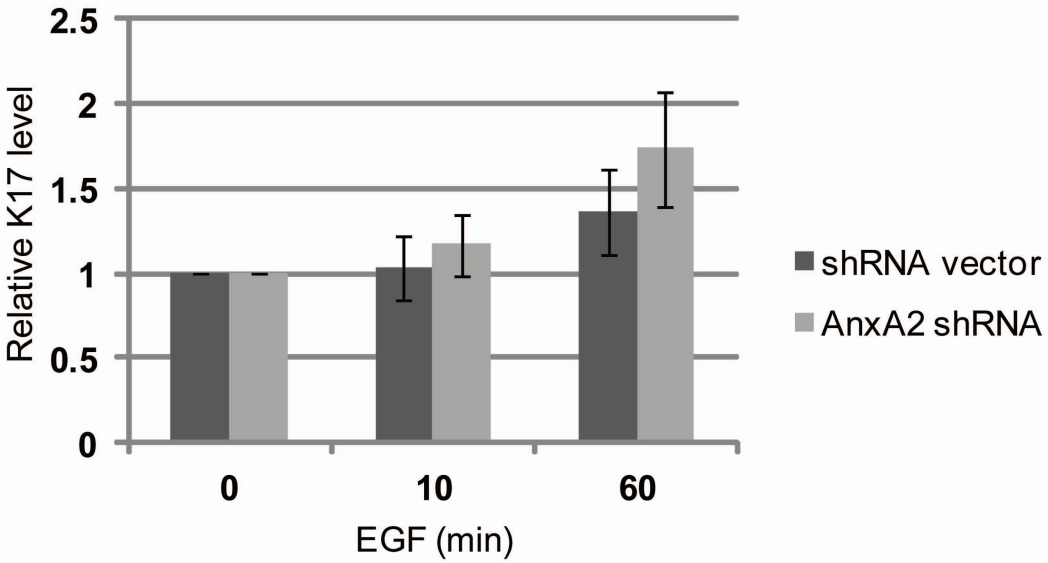

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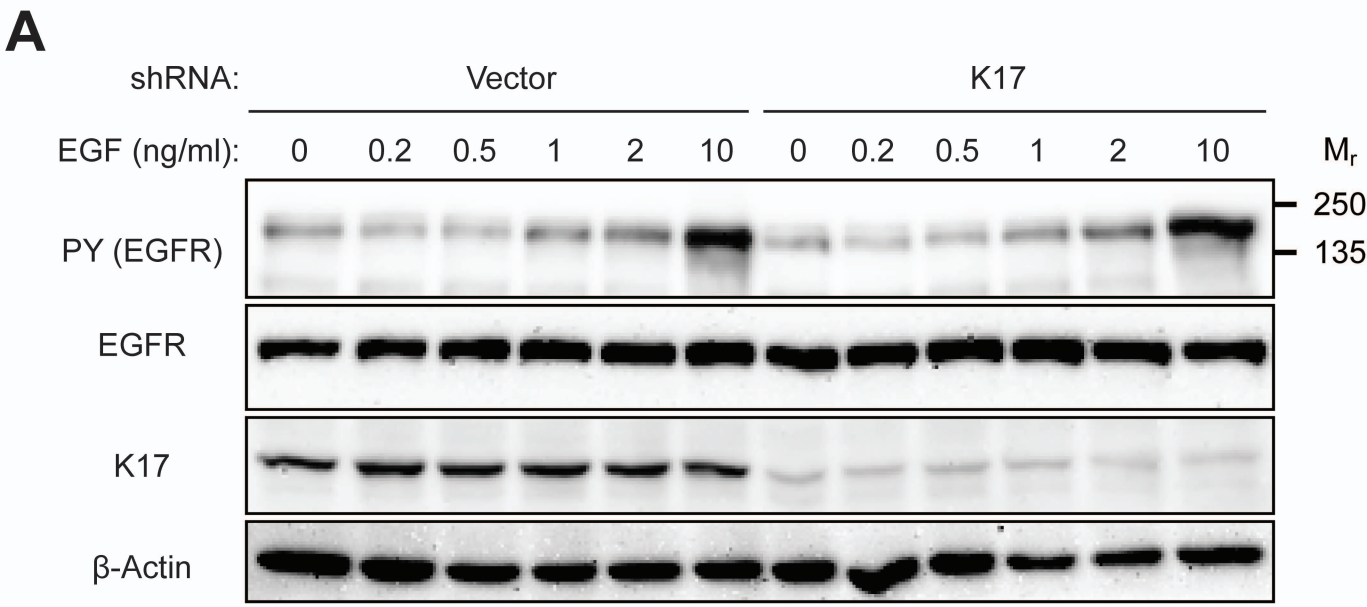
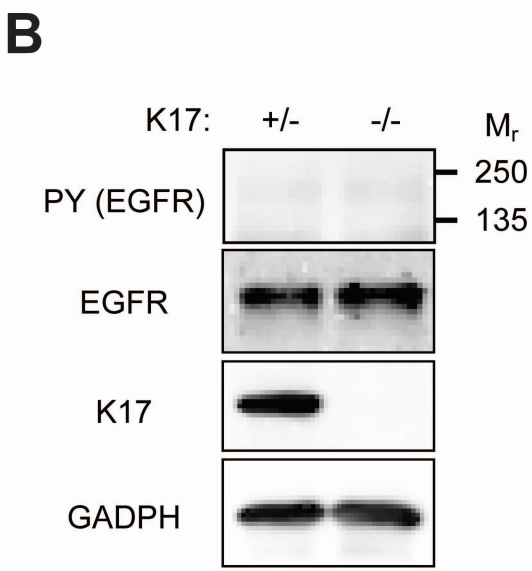
Supplemental Figure S7





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