Chung et al., http://www.jcb.org/cgi/content/full/jcb.201408026/DC1

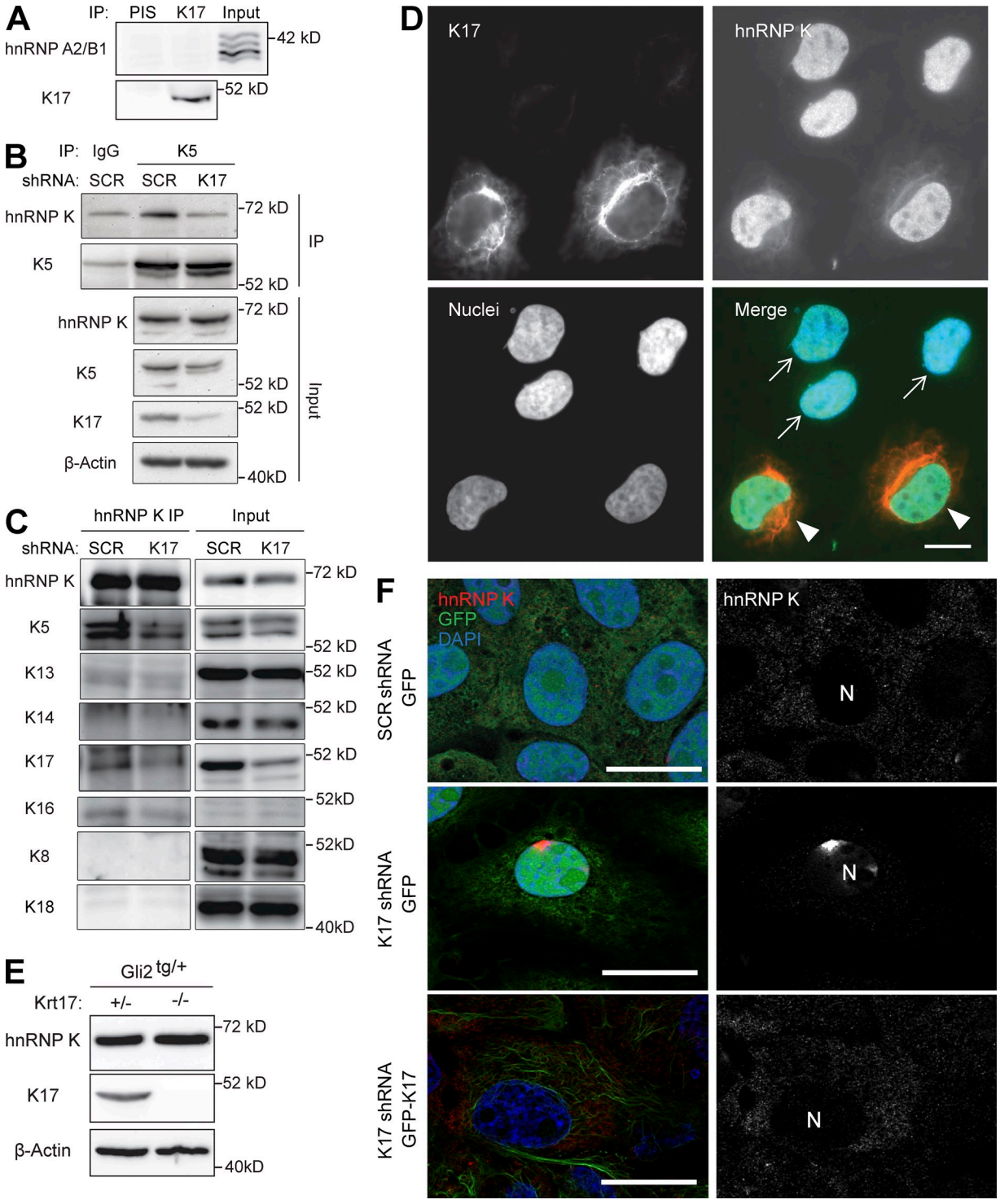


Figure S1. **Specificity of the K17–hnRNP K interaction and K17-dependent hnRNP K localization.** (A) Co-IP of K17 and hnRNP A2/B1. IP was performed in A431 cells with anti-K17 or preimmune serum (PIS) as a control. Immunoblotting was performed with antibodies against the indicated proteins. (B) Co-IP of K5 and hnRNP K. IP with anti-K5 antibody or IgG control was performed in A431 cells stably expressing SCR or *KRT17* shRNA. Immunoprecipitates (IP) or inputs were subjected to SDS-PAGE and immunoblotting was performed with antibodies against the indicated proteins. (C) Co-IP of hnRNP K and keratins. IP with anti–hnRNP K antibody was performed in A431 cells stably expressing SCR or *KRT17* shRNA. Immunoprecipitates (IP) or inputs were subjected to SDS-PAGE and immunoblotting was performed with antibodies against the indicated proteins. (D) Immunostaining of hnRNP K (green) and K17 (red) in HeLa cells transduced with *KRT17* shRNA. Nuclei (N) are shown in blue. Arrows indicate cells expressing *KRT17* shRNA and arrowheads indicate cells that did not become transduced with *KRT17* shRNA. Bar, 10 µm. (E) Cell lysates from HeLa cells stably expressing SCR or *KRT17* shRNA were subjected to SDS-PAGE, and immunoblotting was performed with antibodies against the indicated proteins. (F) Immunostaining of hnRNP K (red) in A431 SCR or *KRT17* shRNA cells transduced with GFP or GFP-K17 (green). Nuclei (N) are shown in blue. Bars, 10 µm.

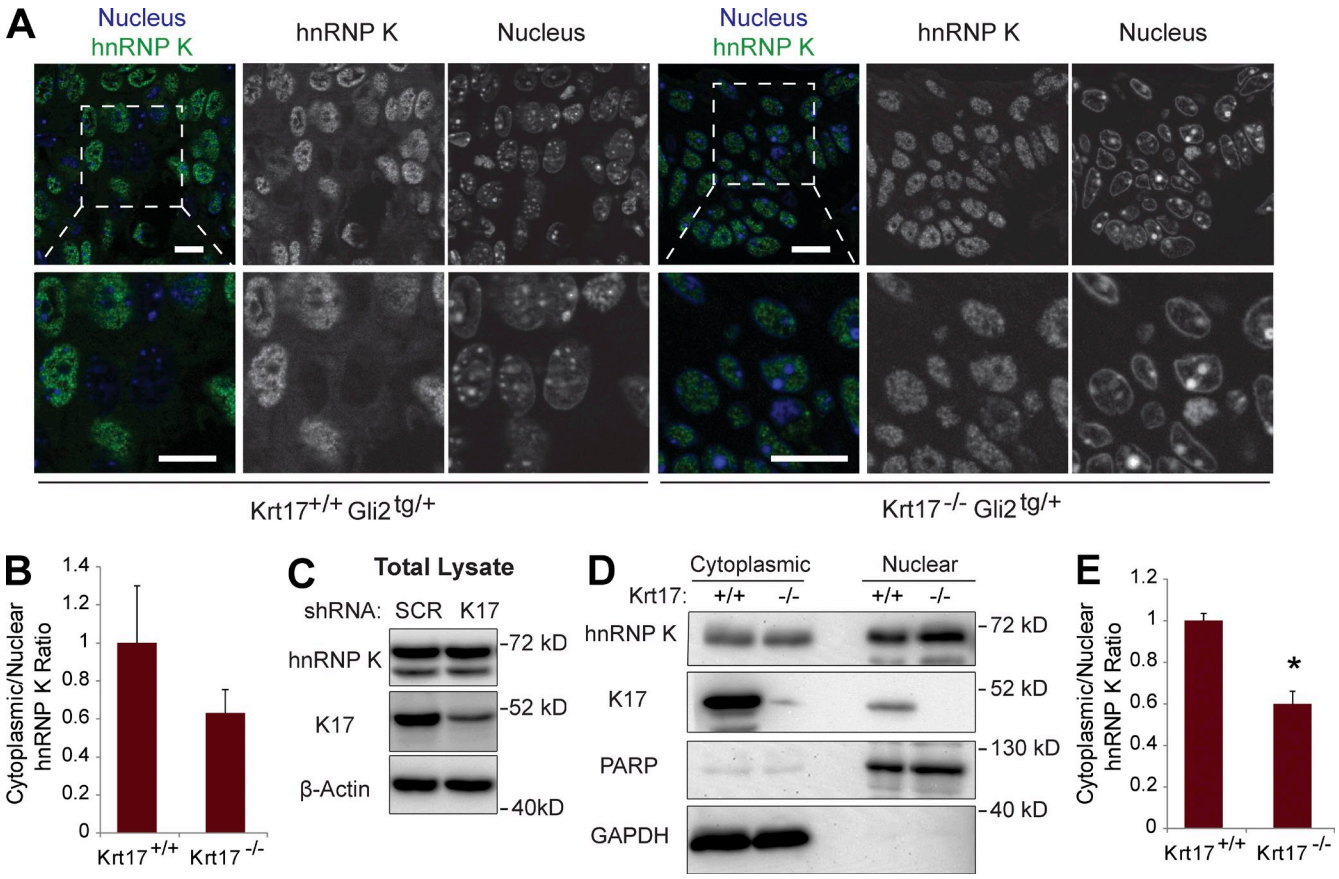


Figure S2. **K17 regulates the cytoplasmic localization of hnRNP K in keratinocytes from *Gli2*$^{tg/+}$ mice.** (A) Immunostaining for hnRNP K (green) in ear lesions from P80 *Krt17*$^{+/+}$ or *Krt17*$^{-/-}$ *Gli2*$^{tg/+}$ mice. Nuclei are shown in blue (Hoechst 33342). Bars, 5 µm. (B) Cytoplasmic-to-nuclear ratios of hnRNP K in A were quantitated using ImageJ, and ratios were normalized to *Krt17*$^{+/+}$ *Gli2*$^{tg/+}$ tissues. Data from three experimental repeats (n = 15) are represented as mean ± SEM (error bars). (C) Cell lysates from primary keratinocytes isolated from *Krt17*$^{+/-}$ or *Krt17*$^{-/-}$ *Gli2*$^{tg/+}$ newborn pups were subjected to SDS-PAGE, and immunoblotting was performed with antibodies against the indicated proteins. (D) Subcellular fractionation of hnRNP K in primary keratinocytes isolated from ear lesions of P60 *Krt17*$^{+/+}$ or *Krt17*$^{-/-}$ *Gli2*$^{tg/+}$ mice. Immunoblotting was performed with antibodies against the indicated proteins. PARP was used as a control for the nuclear fraction while GAPDH was used for the cytoplasmic fraction. (E) Signal intensities of hnRNP K bands from D were quantitated and shown as the cytoplasmic-to-nuclear hnRNP K ratio. Data from four experimental repeats were normalized to *Krt17*$^{+/+}$ *Gli2*$^{tg/+}$ cells and are represented as mean ± SEM (error bars). *, $P < 0.04$.

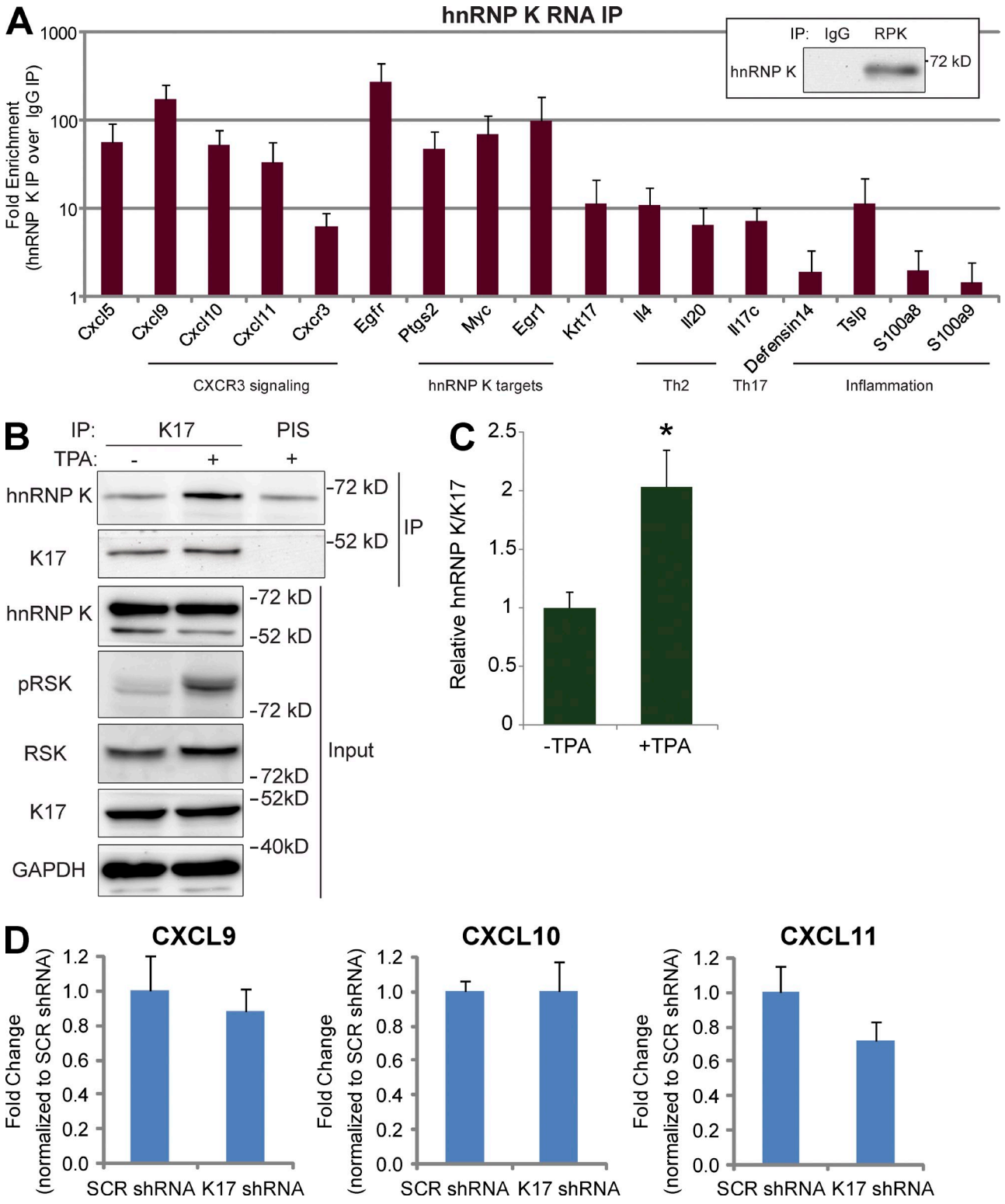
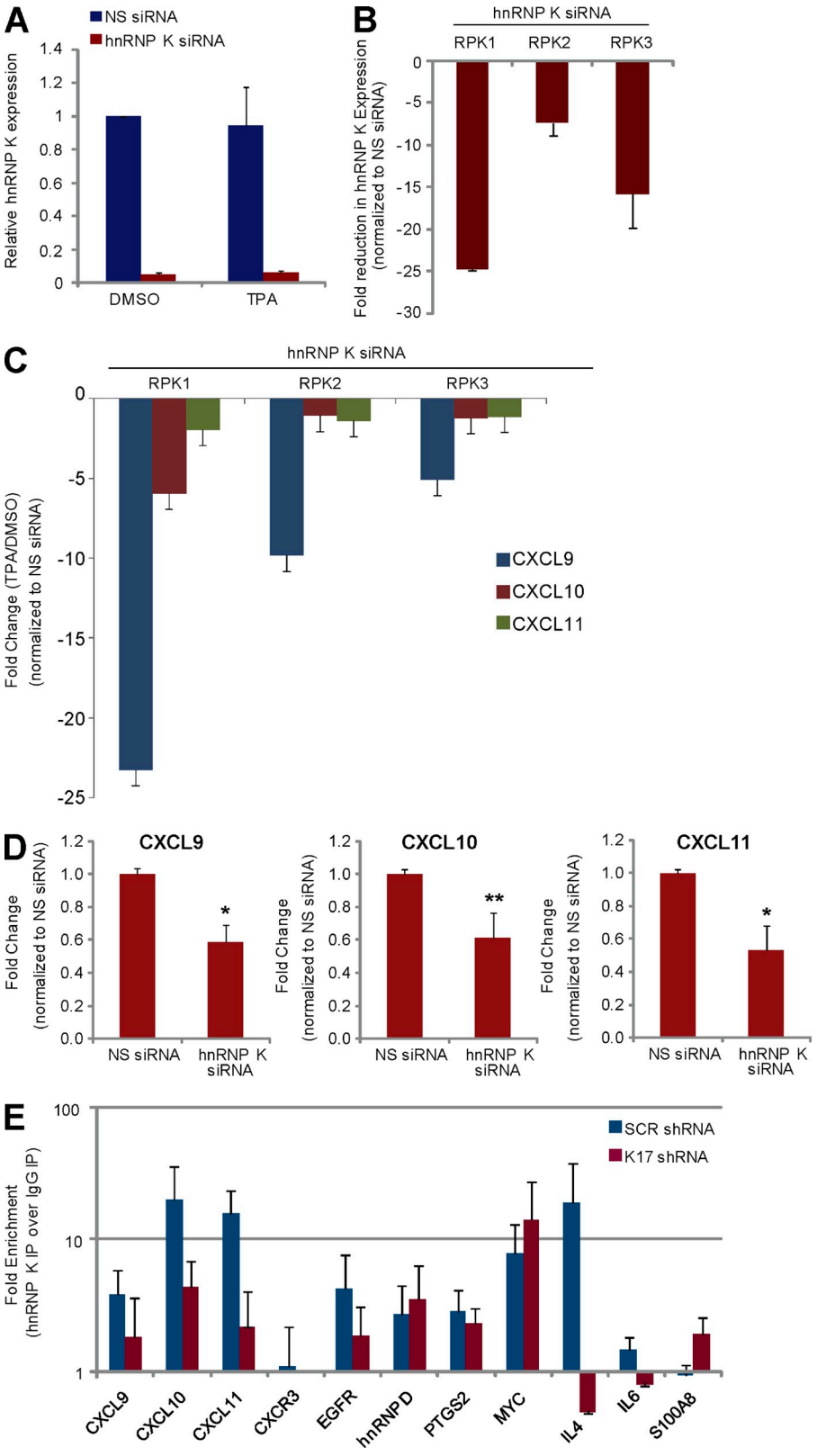


Figure S3. **hnRNP K–bound transcripts in mouse keratinocytes and TPA-induced hnRNP K's interaction with K17.** (A) RNA IP with anti–hnRNP K antibody or IgG control was performed in primary keratinocytes isolated from *Krt17*$^{+/-}$ *Gli2*$^{tg/+}$ newborn pups. mRNA levels of the indicated genes from immunoprecipitates were measured using qRT-PCR. Fold enrichment normalized to IgG control is shown in a log scale. Data from three experimental repeats are represented as mean ± SEM (error bars). (inset) Immunoblot showing hnRNP K (RPK) pull-down. IP (IP) with anti–hnRNP K (RPK) antibody or IgG was performed. Immunoblotting was performed with an hnRNP K antibody. (B) Co-IP of K17 and hnRNP K. IP with anti-K17 antibody or preimmune serum (PIS) control was performed in A431 cells treated with 200 nM TPA or DMSO for 30 min. Immunoprecipitates (IP) or inputs were subjected to SDS-PAGE and immunoblotting were performed with antibodies against the indicated proteins. (C) Signal intensities of hnRNP K and K17 bands from K17 immunoprecipitates in B were quantitated and shown as the hnRNP K–to–K17 ratio. Data from three experimental repeats were normalized to DMSO control and are represented as mean ± SEM (error bars). *, $P < 0.002$. (D) mRNA levels for the indicated genes from A431 cells stably expressing SCR or *KRT17* shRNA under basal condition were measured using qRT-PCR. Data from three experimental repeats ($n = 9$) were normalized to SCR shRNA control and are represented as mean ± SEM (error bars).

Figure S4. **TPA-induced expression of CXCR3 ligands is dependent on hnRNP K.** (A) hnRNP K knockdown levels (a complement to Fig. 2 C). A431 cells transfected with hnRNP K (RPK1) or NS siRNAs were treated with 200 nM TPA or DMSO for 1.5 h. hnRNP K mRNA levels were measured using qRT-PCR. Data from three experimental repeats were normalized to NS siRNA DMSO control and are represented as mean ± SEM (error bars). (B) hnRNP K knockdown levels using three independent siRNAs (RPK1, RPK2, and RPK3) targeting different regions of hnRNP K. A431 cells were transfected with hnRNP K or NS siRNAs, and hnRNP K mRNA levels were measured using qRT-PCR. Data from three experimental repeats were normalized to NS siRNA control and are represented as mean ± SEM to show fold reduction in hnRNP K expression. (C) A431 cells were transfected with siRNAs (RPK1, RPK2, and RPK3) against hnRNP K or NS siRNA. Cells were treated with 200 nM TPA or DMSO (vehicle control) for 6 h and mRNA levels of the indicated genes were measured using qRT-PCR. Data from three experimental repeats were normalized to DMSO, and fold reduction relative to NS control ± SEM is shown. (D) mRNA levels of the indicated genes from A431 cells transfected with NS or hnRNP K siRNA (RPK1) under basal condition were measured using qRT-PCR. Data from four experimental repeats (n = 10) were normalized to SCR shRNA control and are represented as mean ± SEM (error bars). *, $P < 0.007$; **, $P < 0.02$. (E) RNA IP with anti–hnRNP K antibody or IgG control was performed in A431 cells stably expressing SCR or *KRT17* shRNA. mRNA levels of the indicated genes from immunoprecipitates were measured using qRT-PCR. Fold enrichment normalized to IgG control is shown in a log scale. Data from three experimental repeats are represented as mean ± SEM (error bars).



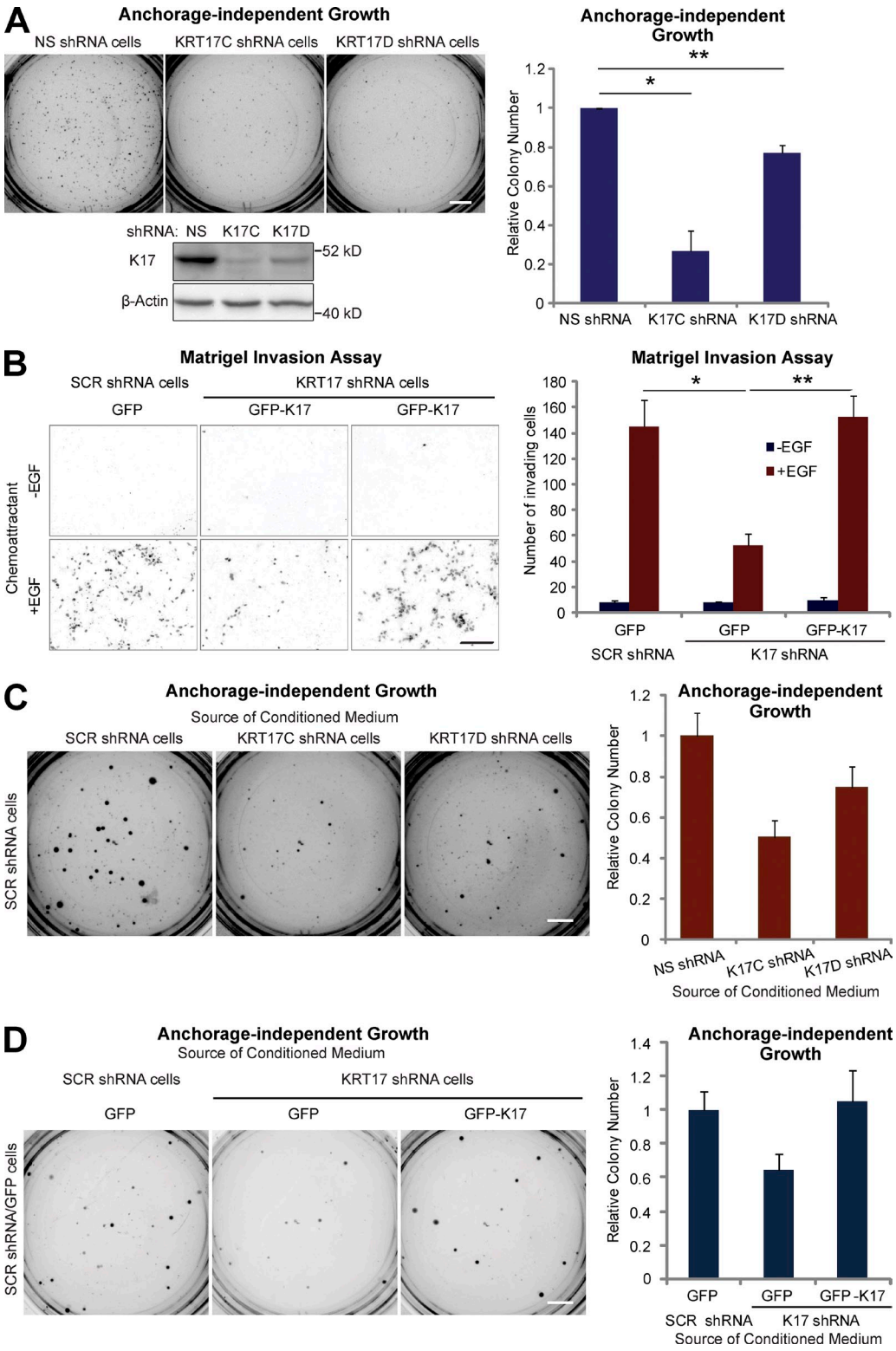


Figure S5. **Validation of anchorage-independent growth and invasion assays.** (A) A431 cells stably expressing NS, *KRT17C*, or *KRT17D* shRNA were grown in soft agar, and phase-contrast images of colonies were taken after 3 wk. Bar, 1 cm. Colony numbers were quantitated using ImageJ, and data from four experimental repeats (each performed in triplicate) were normalized to SCR shRNA and are represented as mean ± SEM (error bars). *, $P < 0.001$; **, $P < 0.05$. Cell lysates of A431 cells stably expressing indicated shRNAs were subjected to SDS-PAGE, and immunoblotting was performed with antibodies against the indicated proteins. (B) A431 SCR or *KRT17* shRNA cells stably expressing GFP or GFP-K17 were subjected to a Matrigel invasion assay with or without 10 ng/ml EGF as a chemoattractant for 48 h. Nuclei of invaded cells were stained with propidium iodide and imaged under a fluorescent microscope. Bar, 100 µm. The numbers of invaded cells were quantitated using ImageJ and data from four experimental repeats (each performed in triplicate) are represented as mean ± SEM (error bars). *, $P < 0.05$; **, $P < 0.003$. (C) Anchorage-independent growth assay using A431 cells stably expressing SCR shRNA in soft agar. Cells were grown in conditioned medium from A431 NS, *KRT17C*, or *KRT17D* shRNA cells. Bar, 1 cm. Colony numbers were quantitated using ImageJ and normalized to those grown in conditioned medium from A431 NS shRNA cells. Data from three experimental repeats (each performed in triplicate) are represented as mean ± SEM (error bars). (D) Anchorage-independent growth assay using A431 SCR shRNA cells stably expressing GFP in soft agar. Cells were grown in conditioned medium from A431 SCR or *KRT17* shRNA cells stably expressing GFP or GFP-K17. Bar, 1 cm. Colony numbers were quantitated using ImageJ and normalized to those grown in conditioned medium from GFP-expressing A431 SCR shRNA cells. Data from three experimental repeats (each performed in triplicate) are represented as mean ± SEM (error bars).