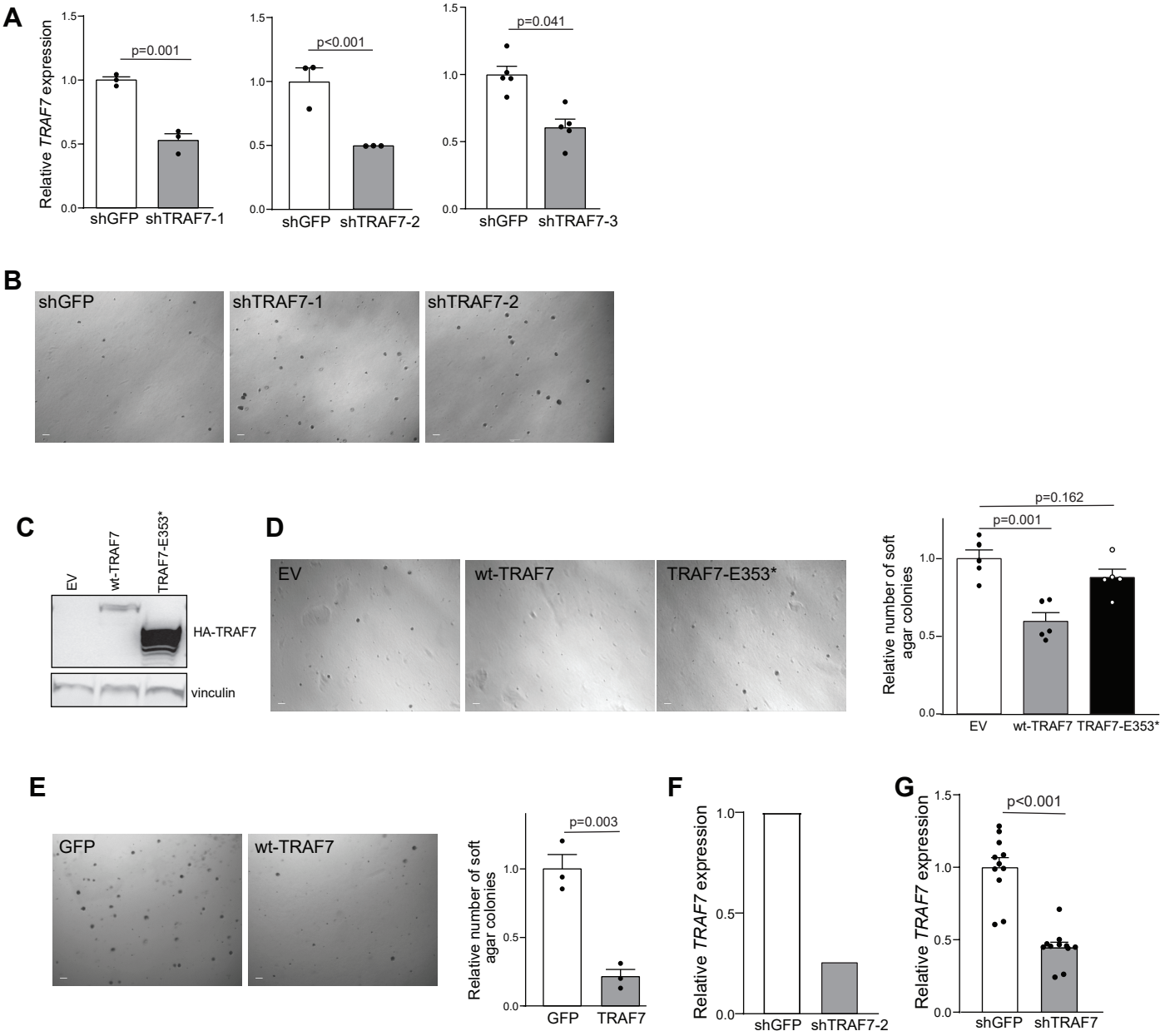


Figure S1. TRAF7 functions as a tumor suppressor in meningeal cells. A, RT-qPCR analysis of *TRAF7* expression in HMCs expressing shRNA targeting *GFP* or *TRAF7*. Values are means ± SEM; n≥3. P value is calculated by a two-sided unpaired Student's t test. **B,** Images of AI colonies of HMCs expressing shRNA against *GFP* or *TRAF7* in soft agar. Scale bar, 100 μm. **C,** Immunoblotting analysis of HMCs expressing an empty vector (EV), wt-*TRAF7*, or *TRAF7-E353** mutants. **D,** Images of AI colonies of HMCs expressing EV, wt-TRAF7, or TRAF7-E353* in soft agar. Scale bar, 100 μm. Relative AI growth is shown as means ± SEM; n≥ 3 P values are calculated by a two-sided Student's t test. **E,** Images of AI growth of CH157-MN cells expressing GFP or wt-TRAF7 in soft agar. Scale bar, 100 μm. Relative AI growth is shown as means ± SEM; n=3. P values are calculated by a two-sided Student' t test. **F,** RT-qPCR analysis of TRAF7 expression of CH157-MN cells expressing sh*GFP* or sh*TRAF7-2*. n=1. **G,** RT-qPCR analysis of *TRAF7* expression of HMCs expressing shGFP or sh*TRAF7-1* and sh*TRAF7-2*. Values are means ± SEM, n>10. P values are from a two-sided unpaired Student's t test.

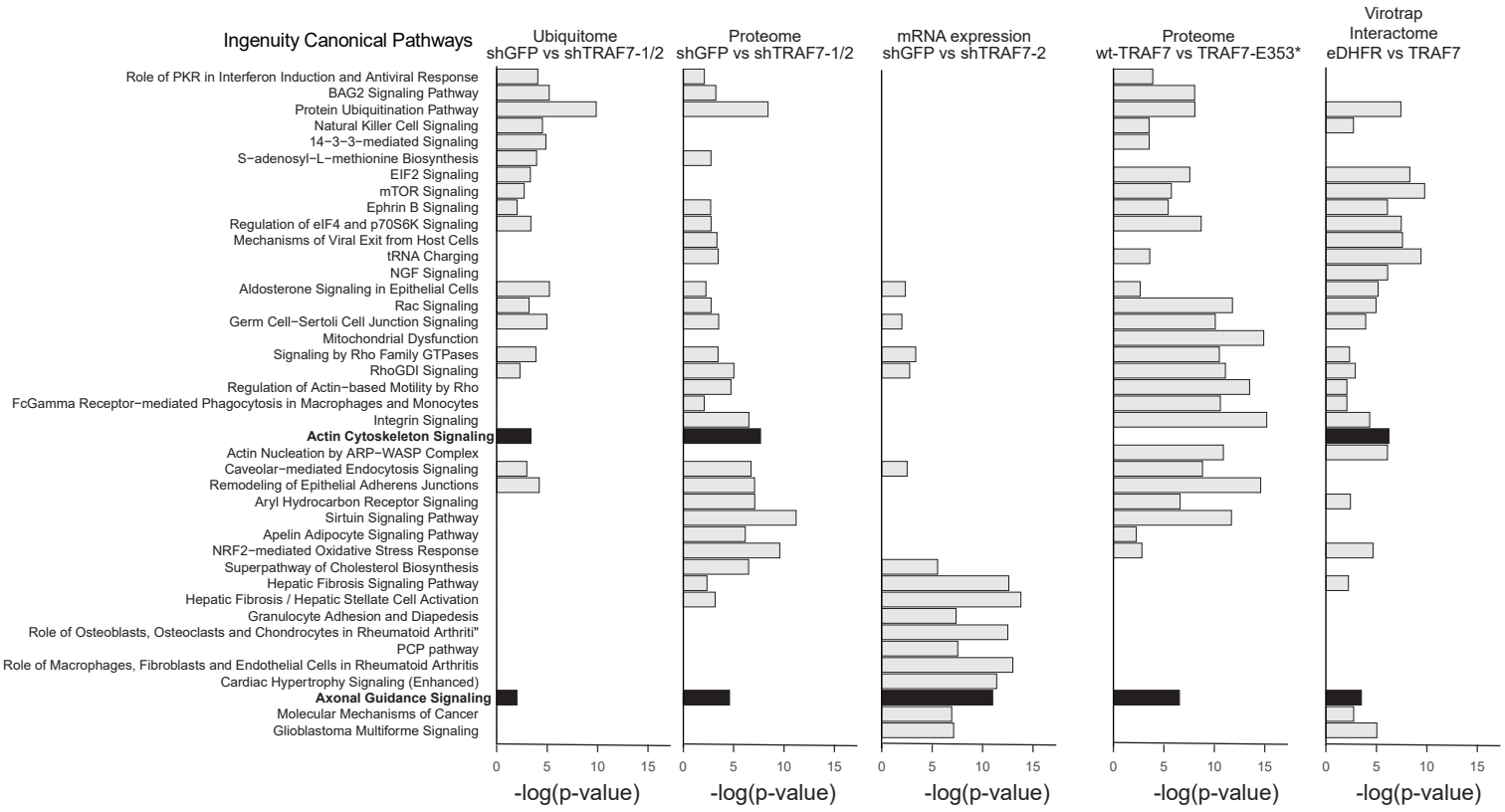


Figure S2. Canonical IPA pathways modulated by TRAF7. Ingenuity Pathway Analysis (IPA) of TRAF7-mediated alterations and TRAF7 interactome. Top 10 significantly enriched pathways for each of the datasets are shown. P value < 0.01 was used to determine the significance. The –log10(P value) was not shown if the pathway was not significantly enriched.

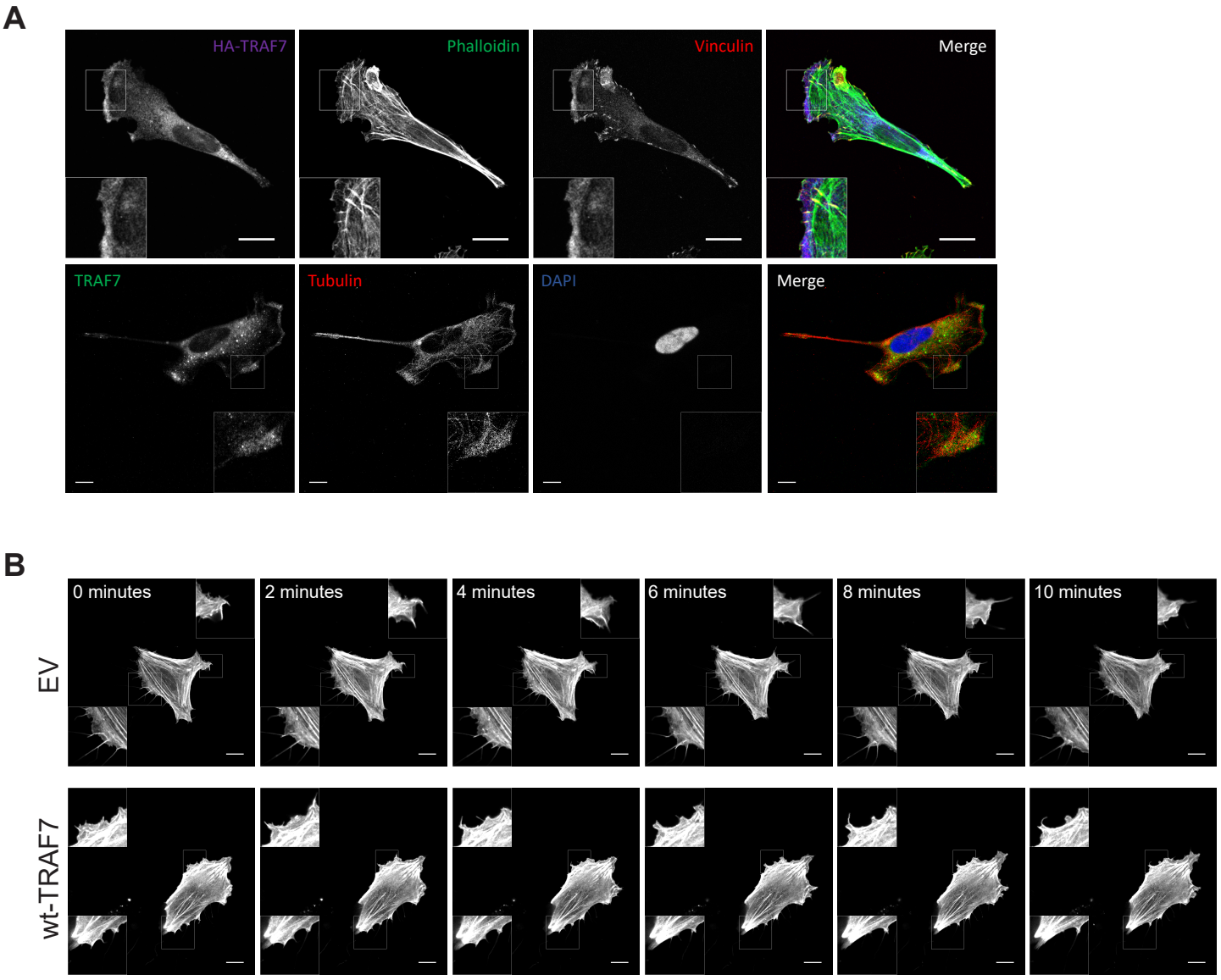


Figure S3. TRAF7-mediated alterations of actin dynamics. A, Immunostaining of HMC expressing HA-TRAF7 with anti-HA, anti-vinculin, or anti-tubulin antibodies, and phalloidin. Scale bar, 10μm. **B,** Human meningeal cells expressing an empty vector (EV) or TRAF7 were transiently transfected with tdTomato-Lifeact-7 and imaged in real time by confocal microscopy for over 10 min. Scale bar, 10 μm.

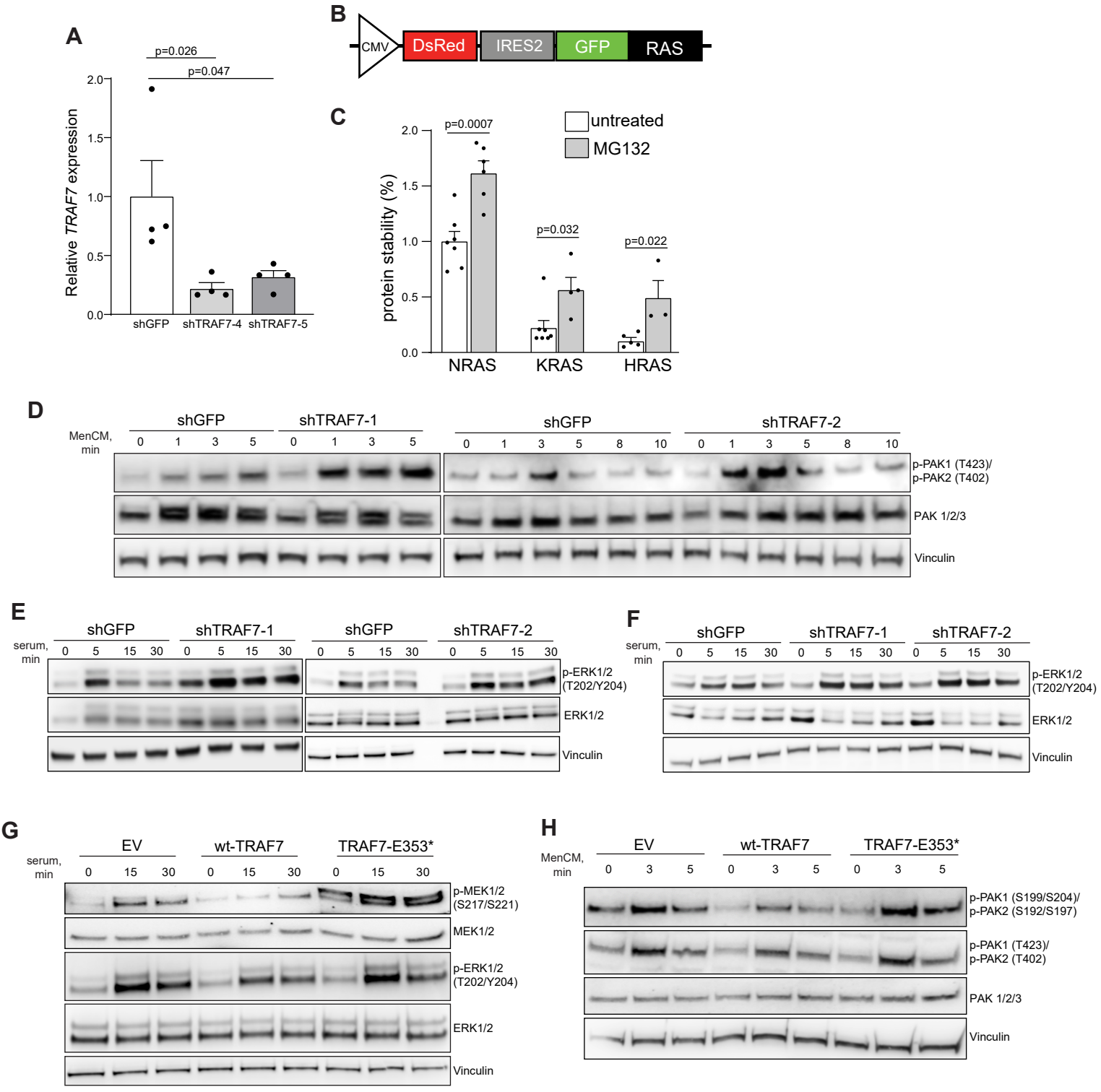


Figure S4. TRAF7 regulates proteostasis and activity of the RAS-like GTPases. A, RT-qPCR analysis of *TRAF7* expression in HEK293T cells expressing shRNAs targeting *GFP* or *TRAF7*. Values are means ± SEM; n=4. P values are from one-way ANOVA. **B,** Schematic representation of the stability reporter system containing DsRed, followed by the IRES sequence and GFP-fused RAS isoforms or CDC42. **C,** Global protein stability (GPS) analysis of relative levels of RAS proteins in HEK293T cells in the absence or presence of MG132 (48 hours). Values are means ± SEM; n ≥ 3. P values are from a two-sided unpaired Student's t test. **D,** Serum-starved HMCs expressing shRNA against *GFP* or *TRAF7* were stimulated with MenCM for the indicated time periods. Immunoblotting analysis using the indicated antibodies. **E,** Serum-starved HMCs expressing shRNA against *GFP* or *TRAF7* were stimulated with 2% serum for the indicated time periods. Immunoblotting analysis using the indicated antibodies. **F,** CH157-MN cells expressing sh*GFP* or sh*TRAF7* were serum-starved overnight, and then stimulated with 10% serum for the indicated time periods. Immunoblotting analysis was performed using the indicated antibodies. **G,** HMCs expressing an empty vector (EV), wt-TRAF7, or TRAF7-E353* mutant were serum-starved overnight, and then stimulated with 2% serum for the indicated time periods. Immunoblotting analysis was performed using the indicated antibodies. **H,** HMCs expressing EV, wt-TRAF7, or TRAF7-E353* mutant were serum-starved overnight, and then stimulated with MenCM, (Meningeal Cell Medium) for the indicated time periods. Immunoblotting analysis was performed using the indicated antibodies.

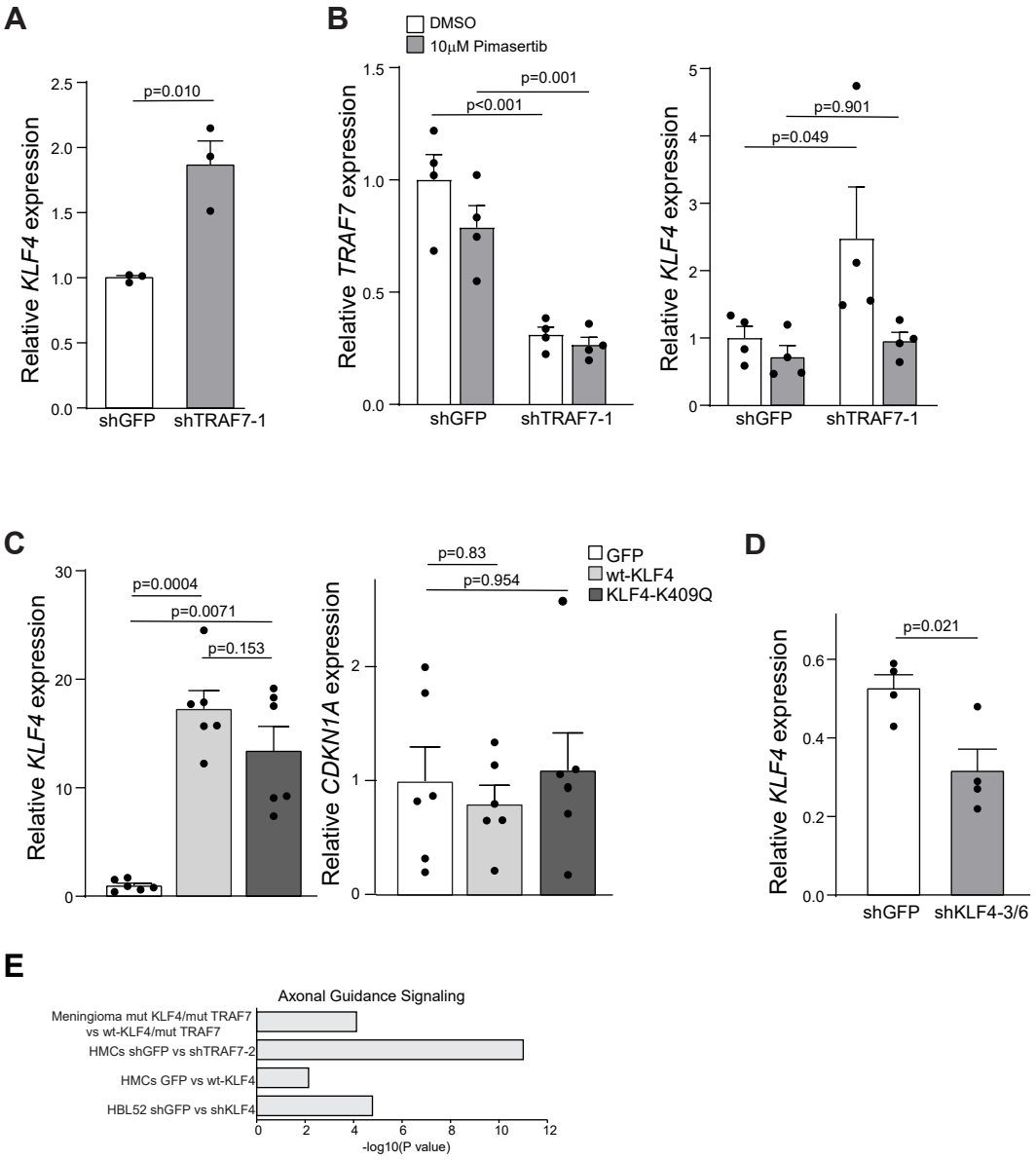
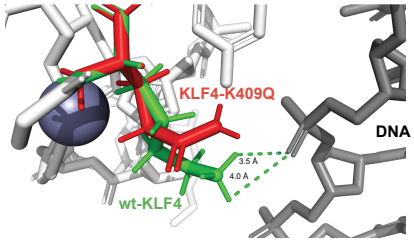


Figure S5. TRAF7 loss of function promotes *KLF4* expression. A, RT-qPCR analysis of *KLF4* expression in human meningeal cells expressing sh*GFP* or sh*TRAF7*-1. Values are mean ± SEM. n=3. P values are from two-sided unpaired Student's t test. **B,** RT-qPCR analysis of *KLF4* and *TRAF7* expression in CH157-MN cells expressing sh*GFP* or sh*TRAF7*-1 treated with DMSO or Pimasertib (10 μM, 48 hours). n=4. P values are from two-way ANOVA. **C,** RT-qPCR analysis of *KLF4* and *CDKN1A* expression in human meningeal cells expressing GFP, wt-KLF4, or KLF4-K409Q. Values are mean ± SEM. n=6. P values are from one-way ANOVA. **D,** RT-qPCR analysis of *KLF4* expression in HBL-52 cells expressing shRNA targeting *GFP* or *KLF4*. Values are mean ± SEM. n=4. P values are from two-sided unpaired Student's t test. **E,** The Axonal Guidance Pathway alterations in the indicated data-sets.

A



B

Sequence Aligment	gRNA sequence targeting KLF4	Indel %	Knock-out score
gSCR-1 vs gKLF4-1	GTGGTGGCGCCCTACAACGG	88 %	78
gSCR-2 vs gKLF4-1	GTGGTGGCGCCCTACAACGG	87 %	77
gSCR-3 vs gKLF4-1	GTGGTGGCGCCCTACAACGG	87 %	77
gSCR-1 vs gKLF4-2	AGCCCGCGTAATCACAAGTG	59 %	54
gSCR-2 vs gKLF4-2	AGCCCGCGTAATCACAAGTG	59%	54
gSCR-3 vs gKLF4-2	AGCCCGCGTAATCACAAGTG	59%	55

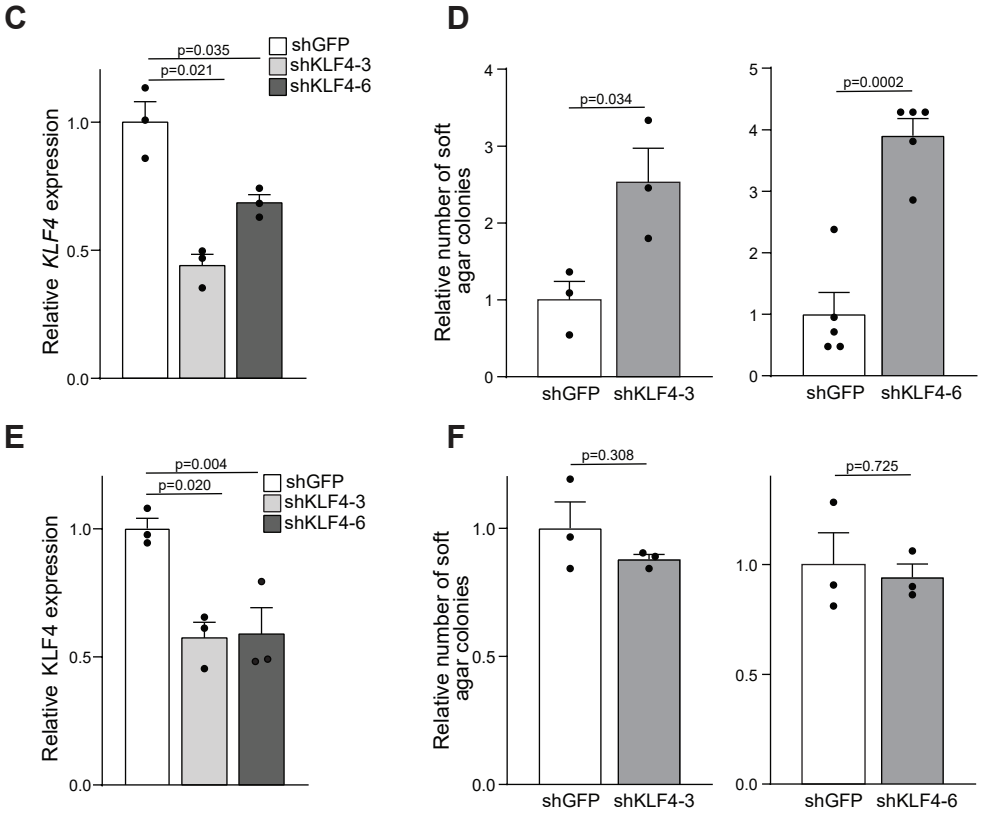


Figure S6. KLF4 promotes transformation of *TRAF7* mutated cells. A, PYMOL in-silico modelling showing interaction between K409 in wt-KLF4 (PDB: 2WBU) or Q409 in KLF4-K409Q mutant and DNA. **B,** Indel efficiency in HMCs expressing the indicated Scramble gRNAs (gSCR) or gRNAs targeting *KLF4* (gKLF4) determined the ICE v2 CRISPR Analysis Tool. **C,** RT-qPCR analysis of *KLF4* expressions in HBL-52 cells expressing shRNA against *GFP* or *KLF4*. Values are mean ± SEM. n=3. P values are calculated by two-sided unpaired Student's t test. **D,** Anchorage independent growth of HBL-52 cells expressing the indicated constructs. Values are means ± SEM. n=3. P values are from two-sided unpaired Student's t test. **E,** RT-qPCR analysis of *KLF4* expressions in HMCs expressing shRNA against *GFP* or *KLF4*. Values are mean ± SEM. n=3. P values are calculated by two-sided unpaired Student's t test. **F,** Anchorage independent growth of HMCs expressing shRNA against *GFP* or *KLF4*. Values are means ± SEM. n=3. P values are from two-sided unpaired Student's t test.

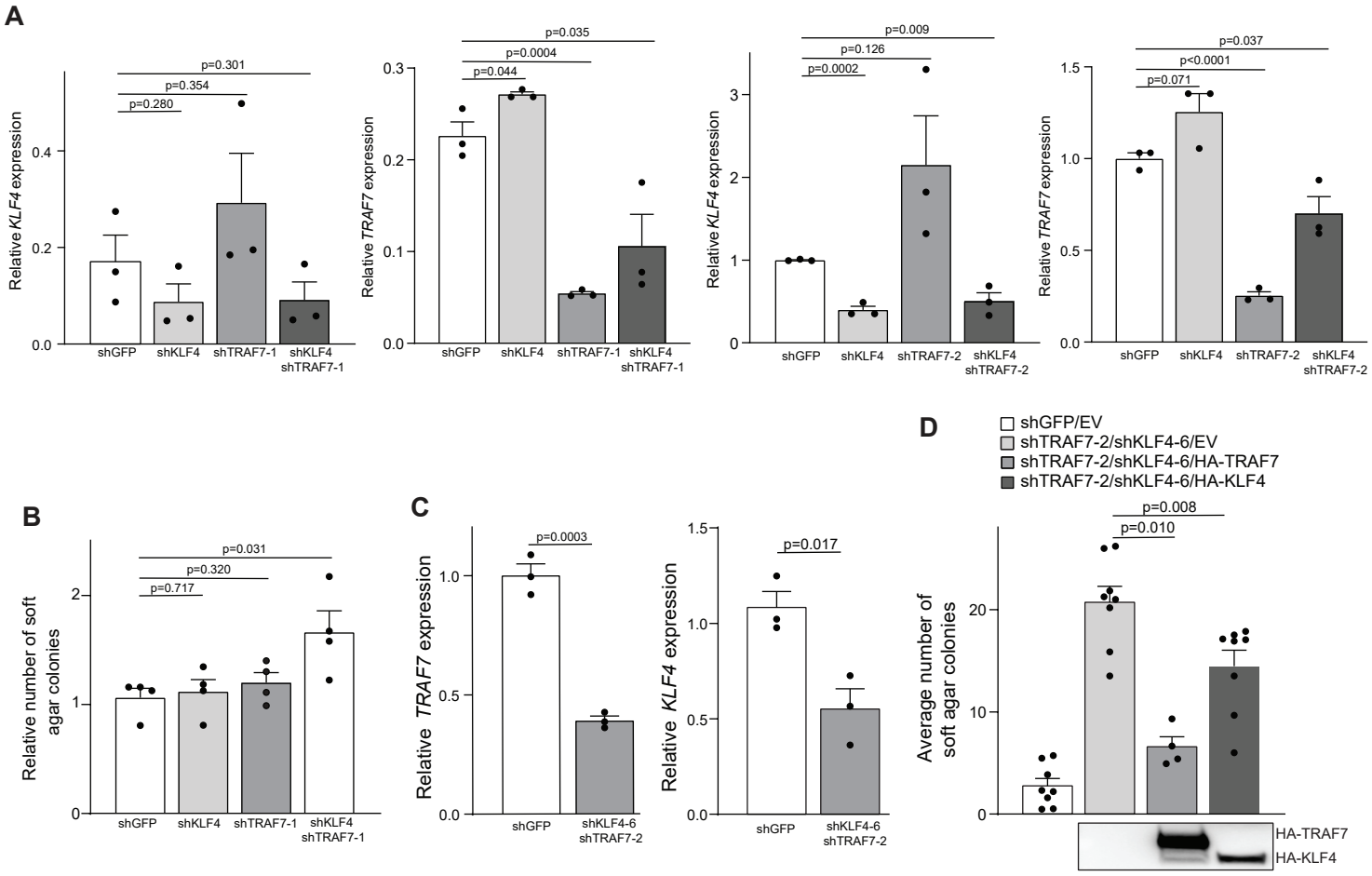


Figure S7. TRAF7 and KLF4 cooperates to promote tumorigenic transformation. A, RT-qPCR analysis of *KLF4* or *TRAF7* expressions in CH157-MN cells expressing the indicated constructs. Values are mean ± SEM. n=3. P values are from two-sided unpaired Student's t test. **B,** Anchorage independent growth of CH157-MN cells expressing the indicated constructs. Values are means ± SEM. n=4. P values are from two-sided unpaired Student's t test. **C,** RT-qPCR analysis of *KLF4* or *TRAF7* expressions in CH157-MN cells expressing the indicated constructs. Values are mean ± SEM. n=3. P values are from two-sided unpaired Student's t test. **D,** Anchorage independent growth of CH157-MN cells expressing the indicated constructs. Values are means ± SEM. n≥4. P values were detected by two-way ANOVA. Immunoblot analysis showed overexpression of HA-TRAF7 or HA-KLF4 using anti-HA antibodies.