

Expanded View Figures

Figure EV1. Validation of the novel pathways of TRF1 regulation.

- A Representative image of the pathways included in Reactome database.
- B Quantification of TRF1 nuclear fluorescence in CHA9-3 lung cancer cells treated with DMSO or structurally different MEK inhibitors for 24 h at 1 μ M. Data are representative of $n = 2$ biological replicates
- C Quantification of TRF1 nuclear fluorescence in CHA9-3 lung cancer cells treated with DMSO or structurally different ERK inhibitors for 24 h at 1 μ M. Data are representative of $n = 2$ biological replicates
- D Western blot images (up) and p-ERK protein levels (down) of CHA9-3 lung cancer cells treated with the indicated compounds for 24 h at 1 μ M. Data are representative of $n = 2$ biological replicates
- E Quantification of TRF1 nuclear fluorescence in CHA9-3 lung cancer cells treated with DMSO or structurally different HSP90 inhibitors for 24 h at 1 μ M. Data are representative of $n = 2$ biological replicates
- F Quantification of TRF1 nuclear fluorescence in CHA9-3 lung cancer cells treated with DMSO or different tubulin agents for 24 h at 1 μ M. Data are representative of $n = 2$ biological replicates.

Data information: Data are represented as mean $\pm$ SEM. Significant differences using unpaired t-test are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Source data are available online for this figure.

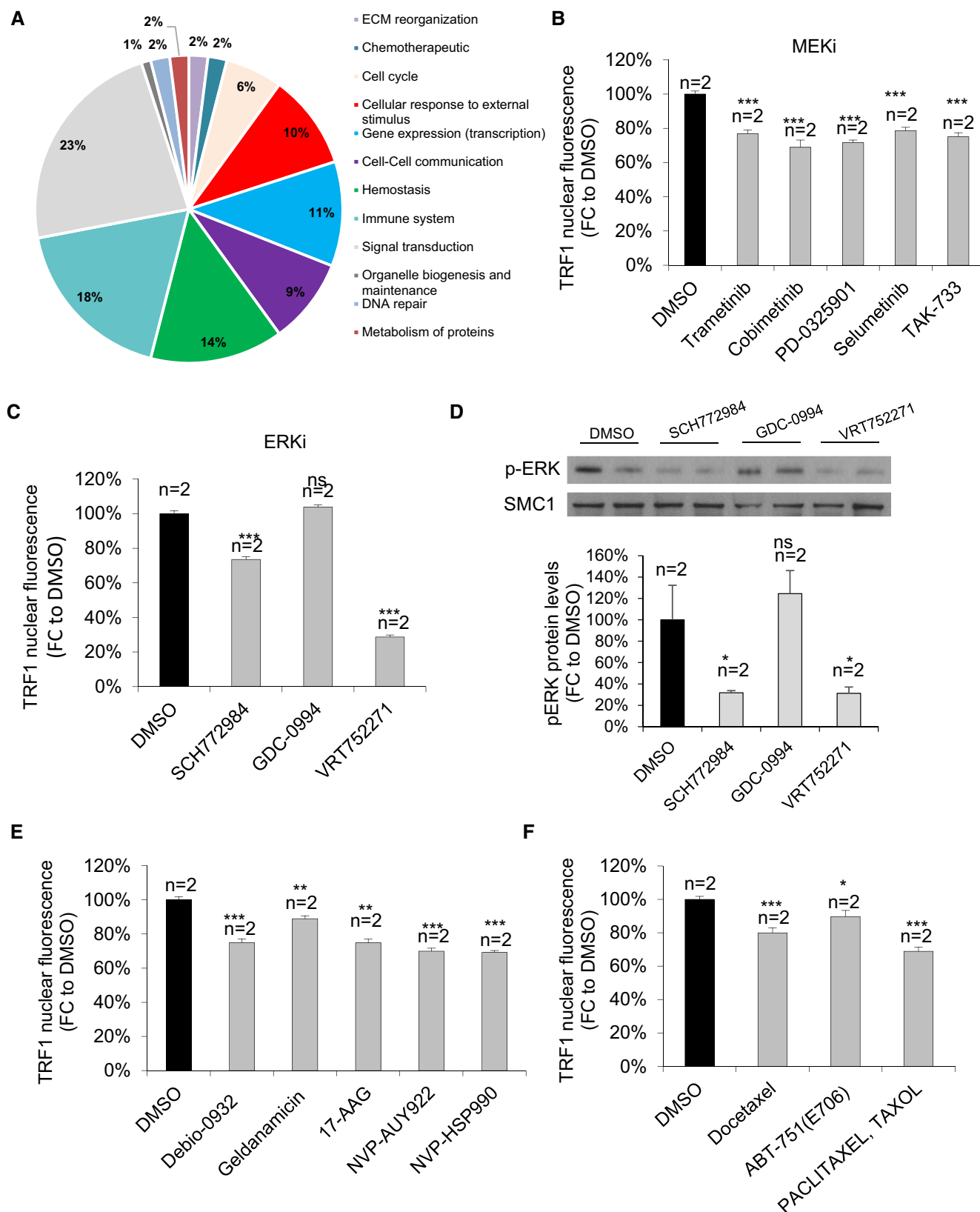


Figure EV1.

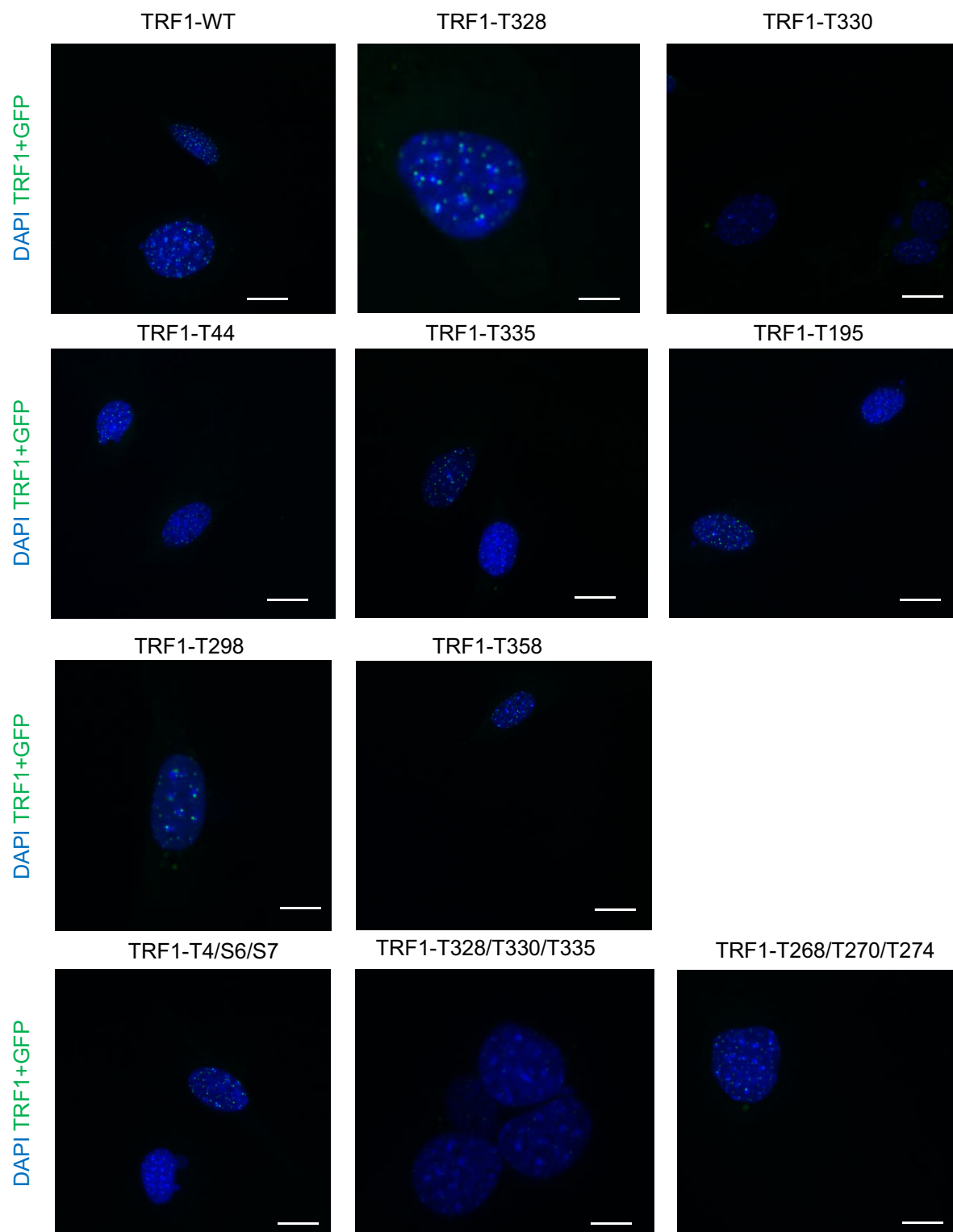


Figure EV2. GFP-tagged TRF1 telomeric foci in MEFs expressing either WT or mutants in ERK-dependent phosphosites of GFP-TRF1.

Representative images of *Trf1*^{Δ/Δ} MEFs transduced with eGFP-*Trf1* WT or mutant alleles as indicated. Scale bars, 5 μm.

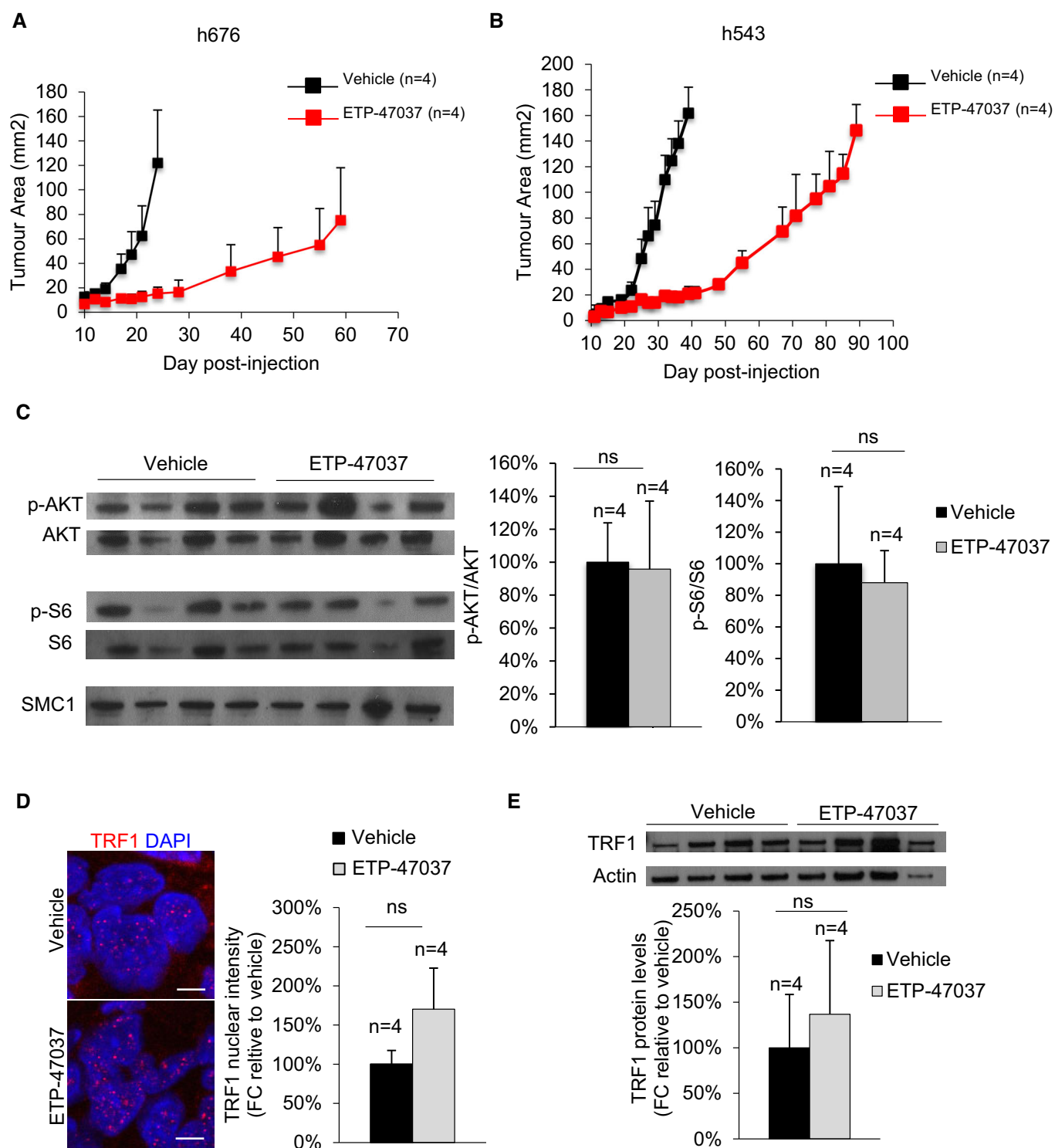


Figure EV3.

Figure EV3. Patient-derived xenografts become resistant to PI3Ki.

- A Longitudinal tumor growth follow-up in ETP-47037 or vehicle-treated mice injected with patient-derived h676 GSCs. Data are representative of $n = 4$ independent tumors.
- B Longitudinal tumor growth follow-up in ETP-47037 or vehicle-treated mice injected with patient derived h543 GSCs. Data are representative of $n = 4$ independent tumors.
- C Western blot images (left) and p-AKT/AKT or pS6/S6 protein levels (right) in vehicle- or ETP-47037-treated tumors. Data are representative of $n = 4$ independent tumors.
- D TRF1 nuclear fluorescence in ETP-47037 or vehicle-treated tumors. Scale bars, 10 μm . Data are representative of $n = 4$ independent tumors
- E Western blot images (left) and TRF1 protein levels (right) in vehicle- or ETP-47037-treated tumors. Data are representative of $n = 4$ independent tumors.

Data information: Data are represented as mean $\pm$ SEM.

Source data are available online for this figure.

Figure EV4. *In vitro* combinatorial studies with the new TRF1 inhibitory compounds.

- A, B Number of spheres formed by patient-derived h676 GSCs 7 days after treatment with the indicated compounds as single agents or in combination. Data are representative of $n = 2$ biological replicates
- C–H Diameter of spheres formed by patient-derived h676 GSCs 7 days after treatment with the indicated compounds as single agents or in combination. n represents biological replicates: in (C) DMSO $n = 42$, PI3Ki $n = 49$, RTKi $n = 56$, Comb $n = 82$; in (D) DMSO $n = 48$, PI3Ki $n = 57$, ERKi $n = 48$, Comb $n = 71$; in (E) DMSO $n = 50$; PI3Ki $n = 49$, MEKi $n = 45$, Comb $n = 47$; in (F) DMSO $n = 39$, PI3Ki $n = 38$, HSP90i $n = 34$, Comb $n = 31$; in (G) DMSO $n = 42$, PI3Ki $n = 35$, Gem $n = 32$, Comb $n = 28$; in (H) DMSO $n = 37$, PI3Ki $n = 35$, Doc $n = 34$, Comb $n = 30$.

Data information: Data are represented as mean $\pm$ SEM. Significant differences using unpaired t -test are indicated by *** $P < 0.001$.

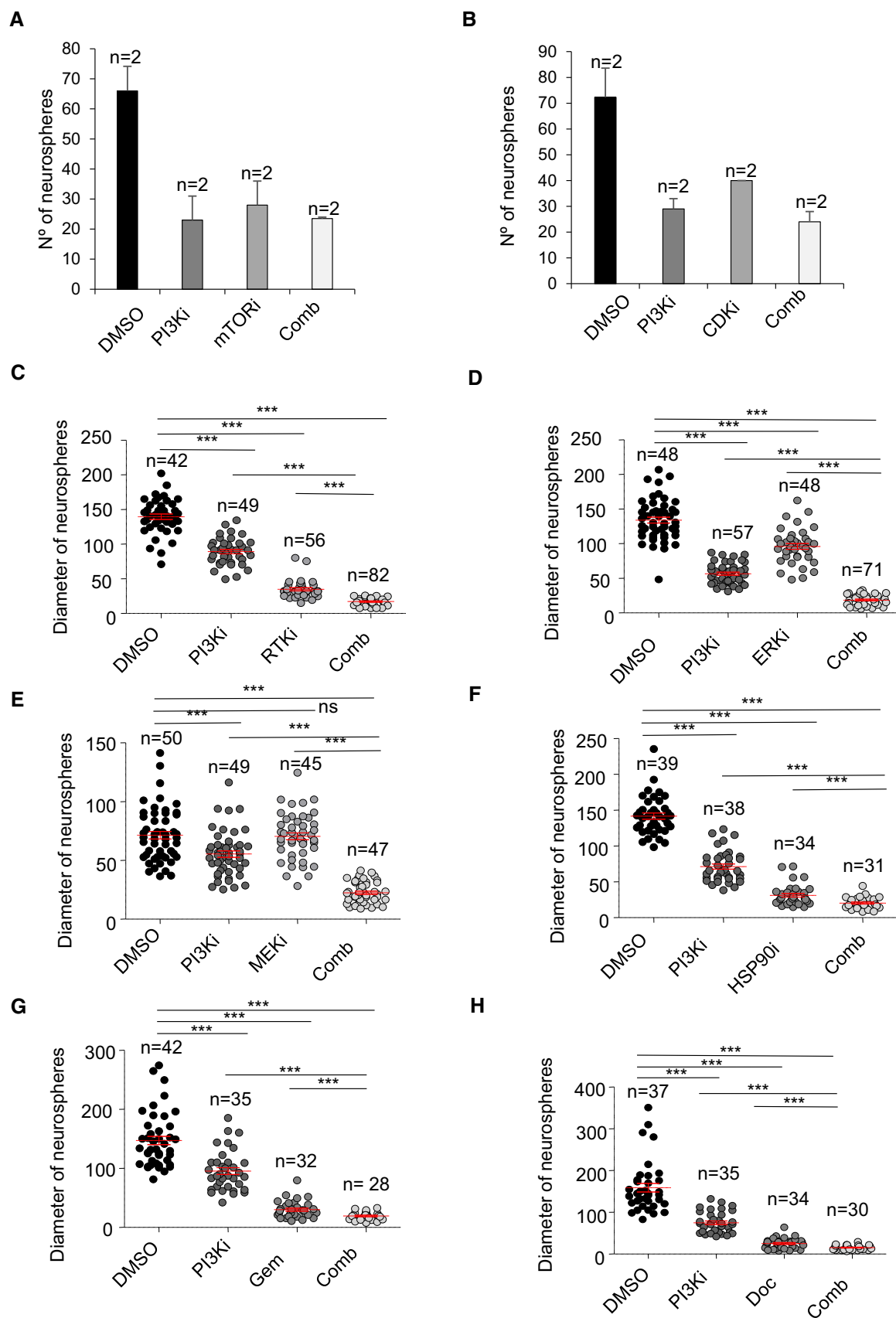


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