

Supplementary Materials

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Blocking ERK-DAPK1 axis attenuates glutamate excitotoxicity in epilepsy

Supplementary Figure Legends

Figure S1. U0126 pretreatment reduces ERK and DAPK1 activity.

Mice (C57BL/6, male/female, 8-12 weeks, n=7 per group) were treated with U0126 (25 mg/kg) and brain tissues were harvested 90 min after administration. **(A-K)** Cortical and hippocampal lysates were subjected to immunoblotting analysis using anti-pSer735-DAPK1, anti-DAPK1, anti-pThr202/Tyr204 ERK, anti-ERK, anti-pSer19-MLC, anti-MLC or anti- β -actin antibodies (**P < 0.01, ***P < 0.001 vs. the control; two-tailed Student's t-test). n.s., not significant (P > 0.05). All the values were combined and are expressed as the mean \pm SD. Experiments were performed in triplicate with at least two mice per group per experiment.

Figure S2. U0126 pretreatment decreases ERK and DAPK1 activity.

Mice (C57BL/6, male/female, 8-12 weeks, n=7 per group) were treated with U0126 (25 mg/kg) and brain tissues were harvested 180 min after administration. **(A-K)** Cortical and hippocampal lysates were subjected to immunoblotting analysis using anti-pSer735-DAPK1, anti-DAPK1, anti-pThr202/Tyr204 ERK, anti-ERK, anti-pSer19-MLC, anti-MLC or anti- β -actin antibodies (**P < 0.01, ***P < 0.001 vs. the control; two-tailed Student's t-test). n.s., not significant (P > 0.05). All the values were combined and are expressed as the mean \pm SD. Experiments were performed in triplicate with at least three mice per group per experiment.

Figure S3. The information of Tat-DM and Tat-s-DM.

(A-B) High performance liquid chromatography report displays the purity (chromatogram) and relative parameters of the synthesized peptide Tat-DM or Tat-s-DM. **(C-D)** Mass Spectrometry assay shows relative parameters of the synthesized peptide Tat-DM or Tat-s-DM.

Figure S4. Tat-DAPK1DM peptide pretreatment blocks ERK and DAPK1 interaction.

Mice (C57BL/6, male/female, 8-12 weeks, n=7 per group) were treated with Tat-DM peptide (10 mg/kg) and brain tissues were harvested 3 or 6 h after administration. **(A-B)**

Hippocampal lysates were immunoprecipitated with non-specific IgM or anti-DAPK1 antibodies and then subjected to immunoblotting analysis using anti-DAPK1 or anti-ERK antibodies ($***P < 0.001$; two-tailed Student's t-test). **(C-H)** Hippocampal lysates were subjected to immunoblotting analysis with anti-pSer735-DAPK1, anti-DAPK1, anti-pThr202/Tyr204 ERK, anti-ERK, or anti- β -actin antibodies ($*P < 0.05$, $**P < 0.01$ vs. the control; two-tailed Student's t-test). n.s., not significant ($P > 0.05$). All the values were combined and are expressed as the mean \pm SD. Experiments were performed in triplicate with at least two mice per group per experiment.

Figure S5. Verification of S735A mutant plasmid.

Flag-DAPK1 or Flag-DAPK1 S735A were transfected to HEK 293T cells for 48 h, and harvested cell lysates were immunoprecipitated with anti-Flag antibody and then subjected to immunoblotting analysis using anti-FLAG, anti-pSer735-DAPK1 or anti- β -actin antibodies.

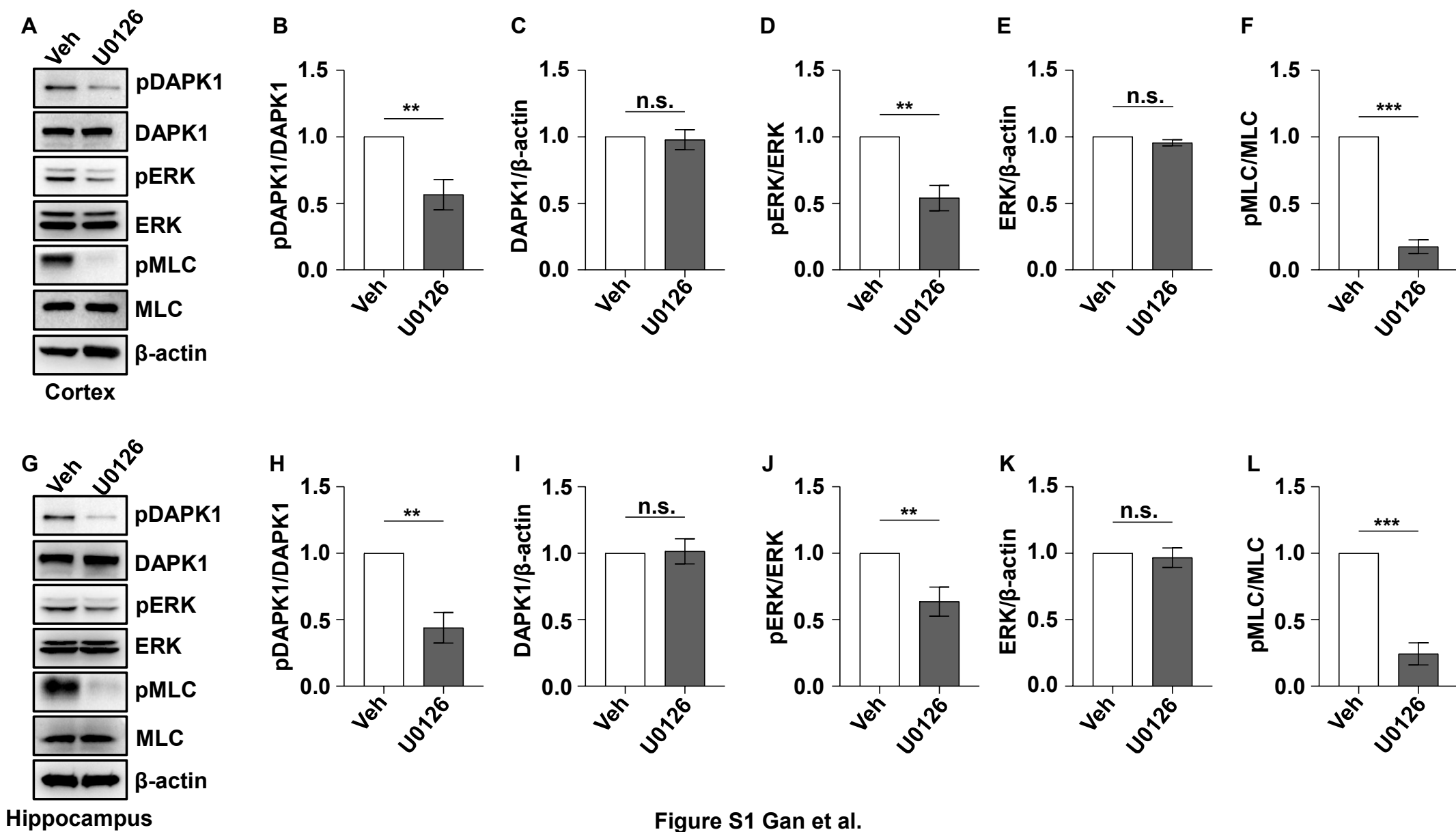


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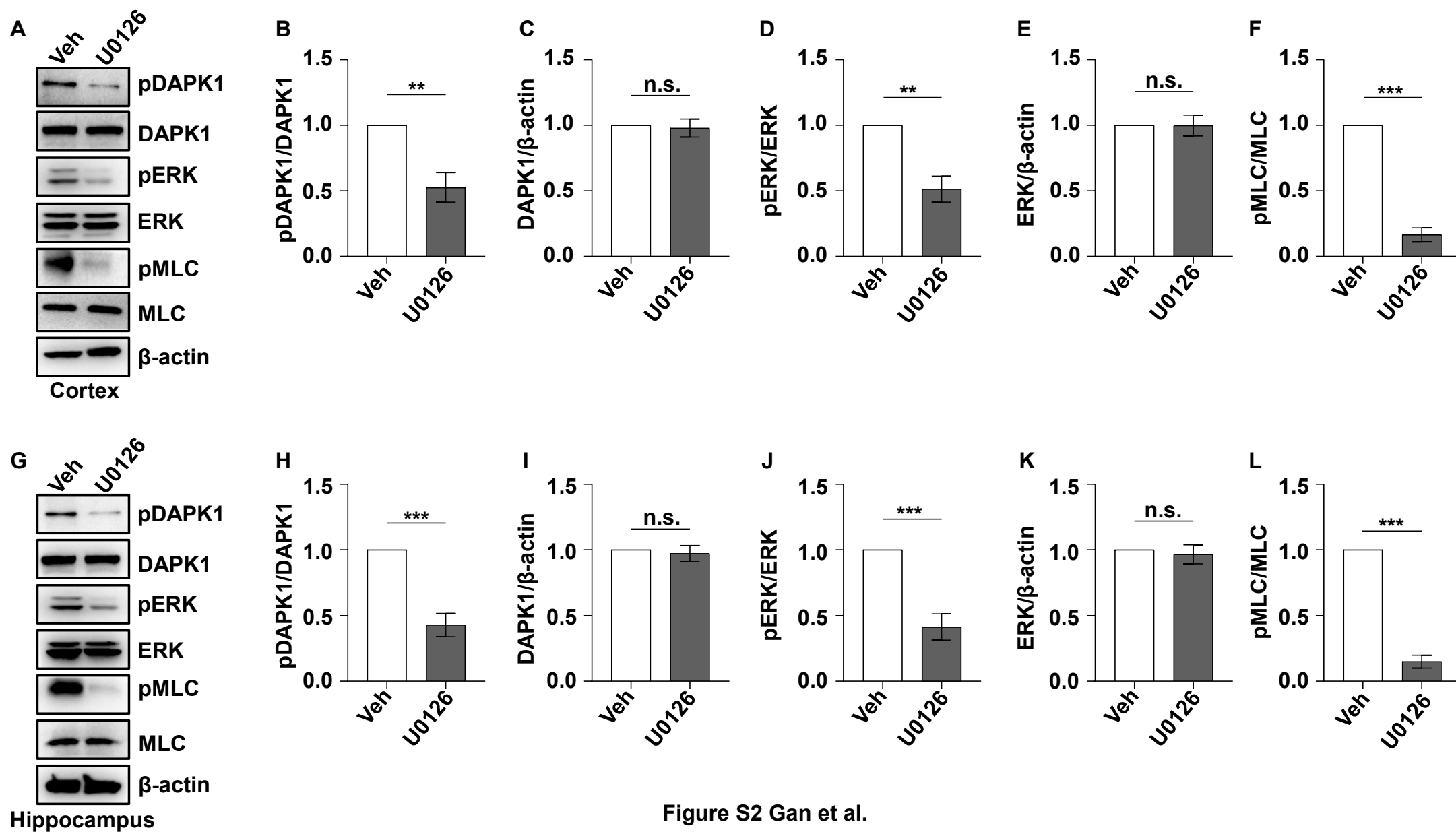
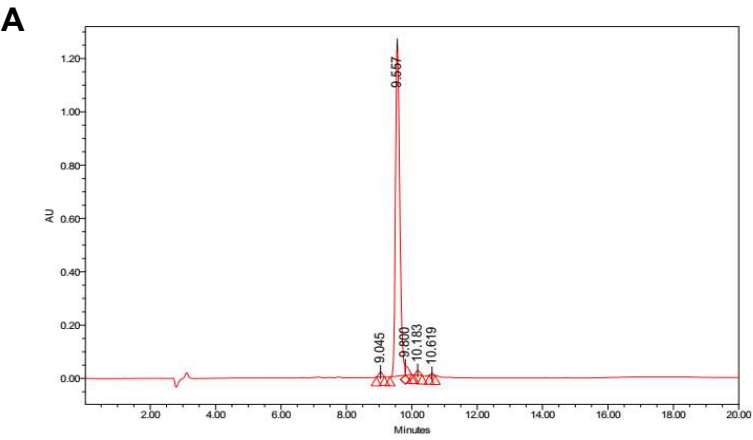
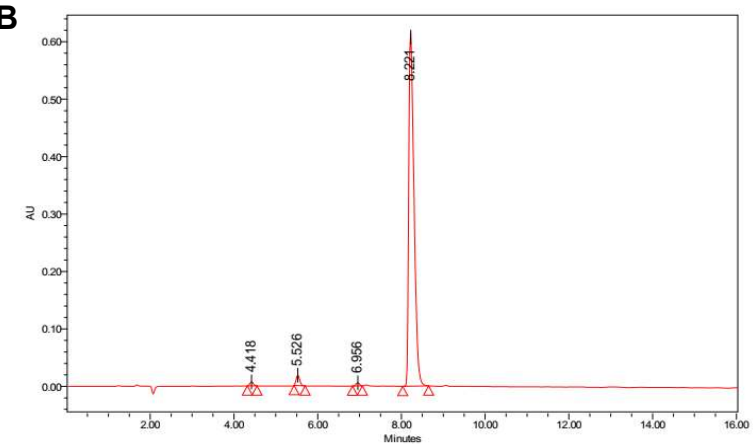


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Processed Channel: 2487Channel 1

Processed Channel	Retention Time (min)	Area	% Area	Height
1 2487Channel 1	9.045	135701	1.13	17961
2 2487Channel 1	9.557	11427221	95.19	1253424
3 2487Channel 1	9.800	248006	2.07	34733
4 2487Channel 1	10.183	146327	1.22	16460
5 2487Channel 1	10.619	47865	0.40	8030



Processed Channel: 2487Channel 1

Processed Channel	Retention Time (min)	Area	% Area	Height
1 2487Channel 1	4.418	41969	0.76	7098
2 2487Channel 1	5.526	109724	1.97	18031
3 2487Channel 1	6.956	32436	0.58	5674
4 2487Channel 1	8.221	537346	96.69	616382

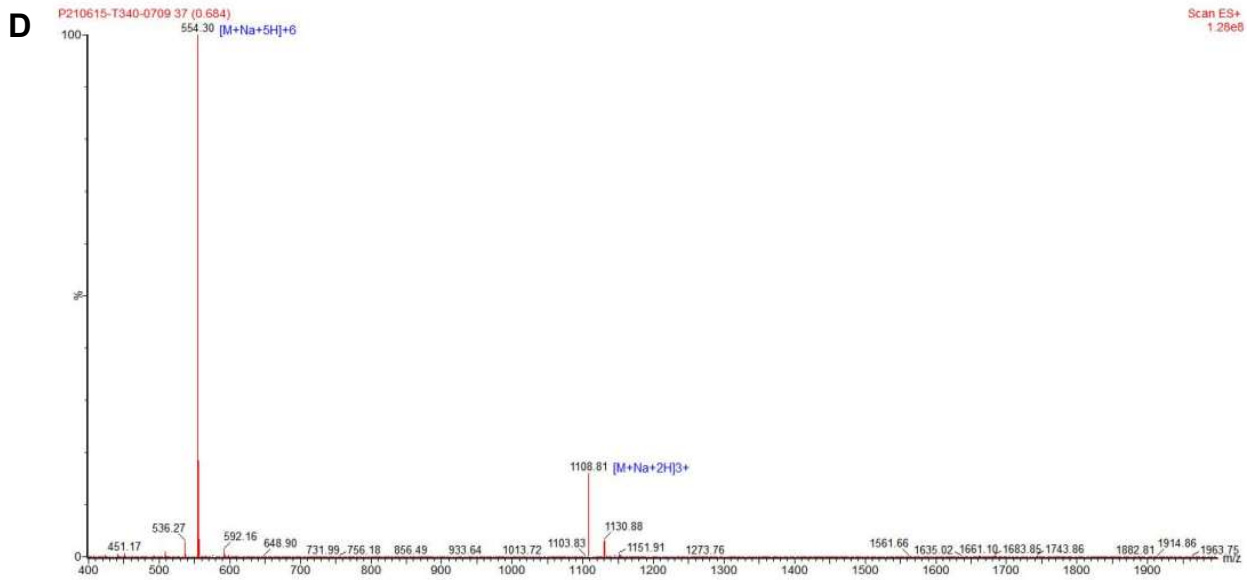
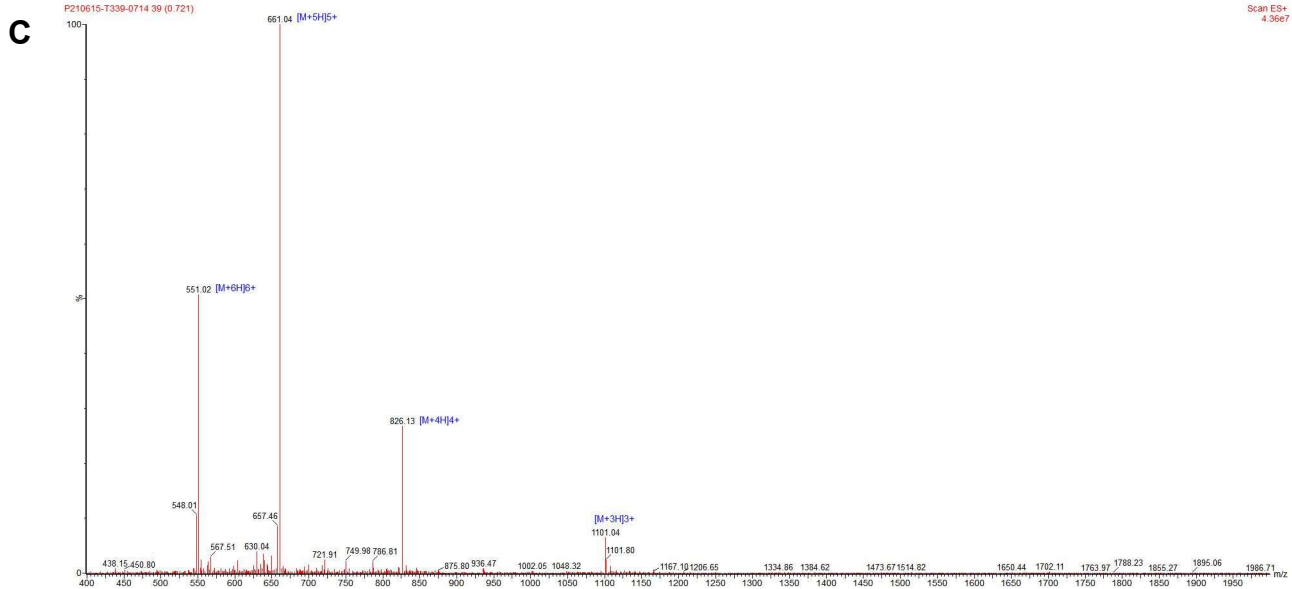


Figure S3 Gan et al.

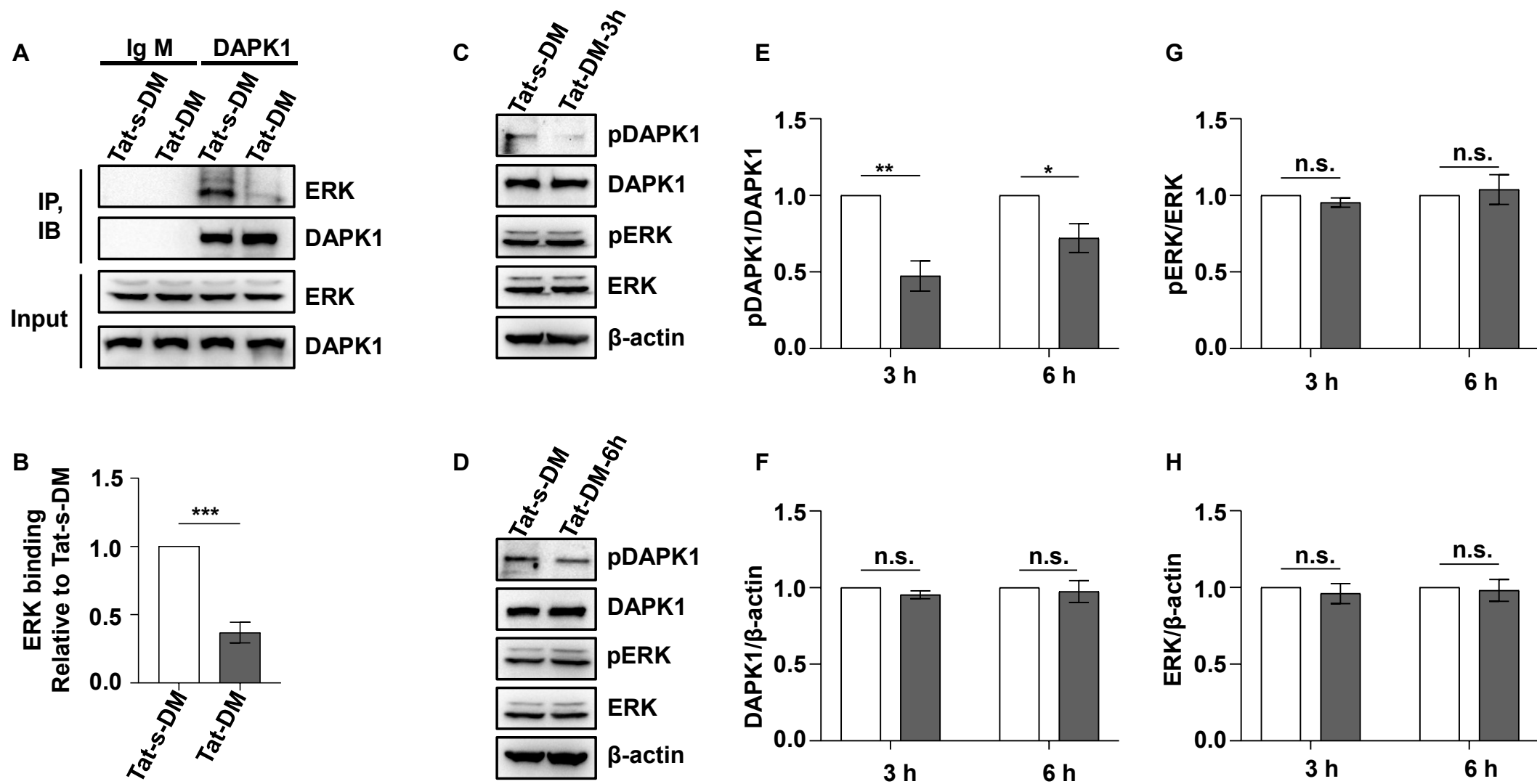


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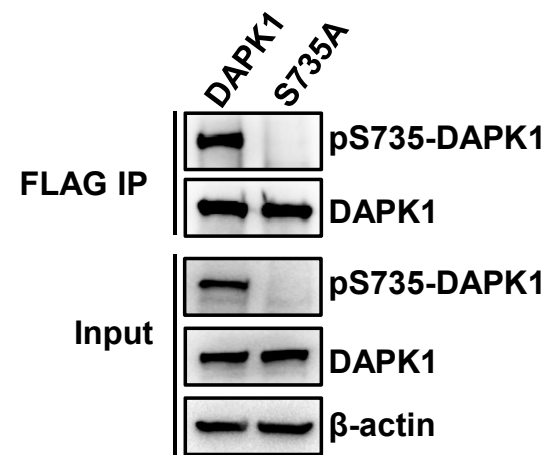


Figure S5 Gan et al.