

Supplementary Materials for
Functional neuronal circuits promote disease progression in cancer

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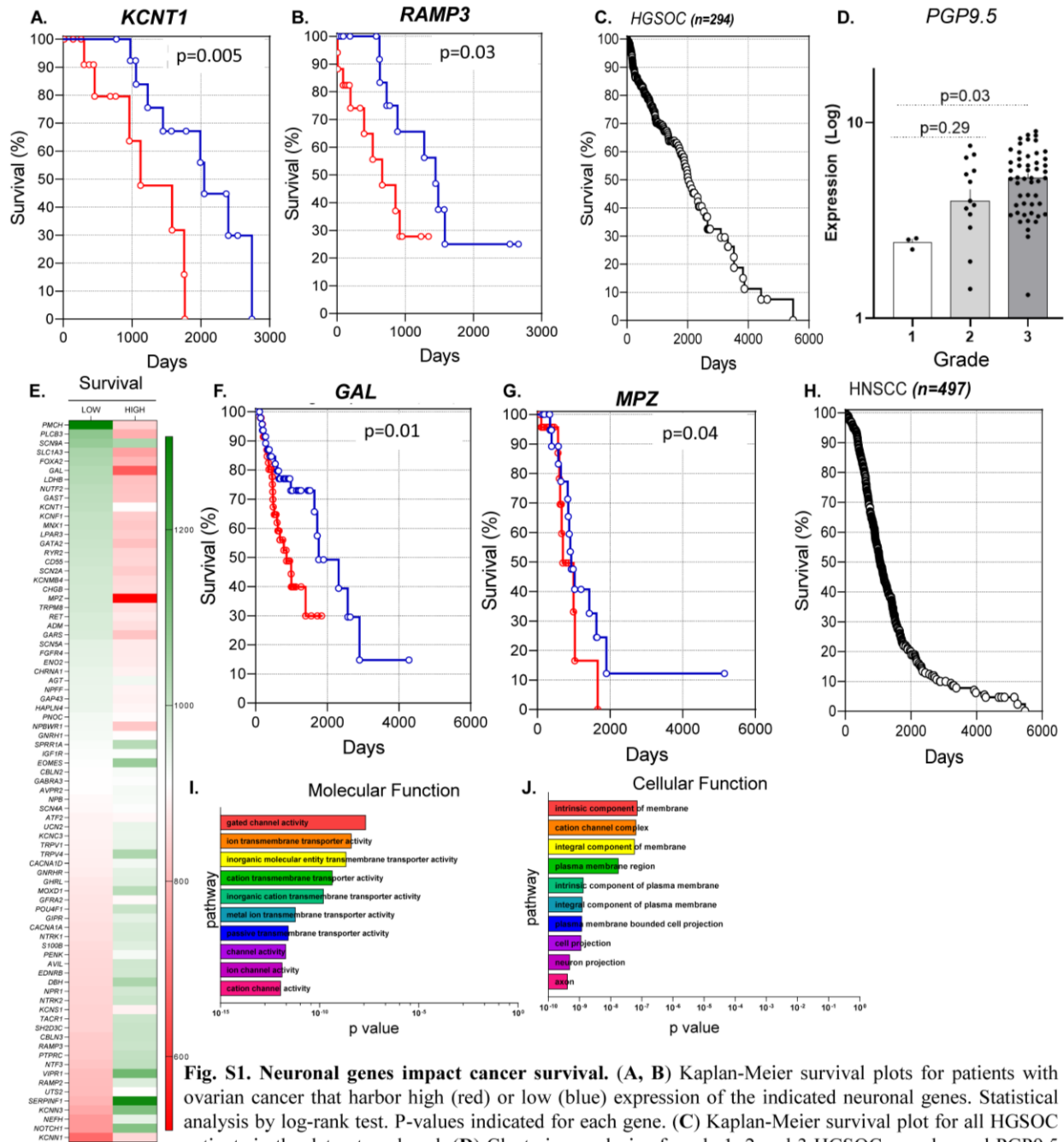


Fig. S1. Neuronal genes impact cancer survival. (A, B) Kaplan-Meier survival plots for patients with ovarian cancer that harbor high (red) or low (blue) expression of the indicated neuronal genes. Statistical analysis by log-rank test. P-values indicated for each gene. (C) Kaplan-Meier survival plot for all HGSOC patients in the dataset analyzed. (D) Clustering analysis of grade 1, 2 and 3 HGSOC samples and PGP9.5 (neuronal gene). Statistical analysis by one-way ANOVA with post-hoc Dunnet. $p=0.29$ (1 vs 2); $p=0.03$ (1 vs 3). (E) Heatmap of neuronal genes expressed by head and neck cancers from OncoLnc, Gepia2 and Oncomine datasets. Correlations between gene expression and patient survival shown. (F, G) Kaplan-Meier survival plots for patients with head and neck cancer that harbor high (red) or low (blue) expression of the indicated neuronal genes. Statistical analysis by log-rank test. P-values indicated for each gene. (H) Kaplan-Meier survival plot for all HNSCC patients in the dataset analyzed. (I, J) GO enrichment analysis of neuronal genes demonstrating significant pathways important for molecular and cellular functions in head and neck cancers.

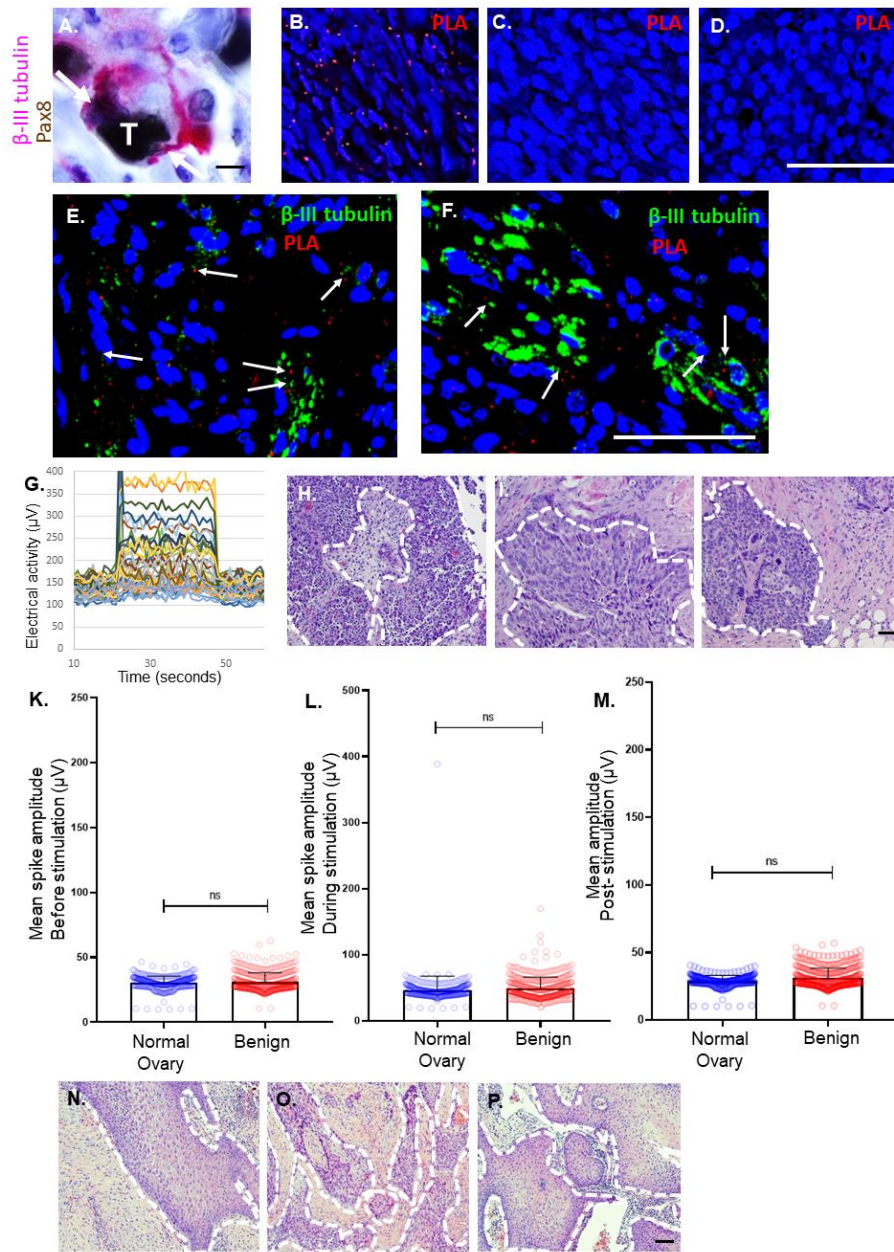


Fig. S2. Tissue controls. (A) Representative photomicrograph bright field image of HGSOC ($n=4$) patient samples double immunohistochemically stained for tumor cells (T, brown, Pax8) and intra-tumoral nerves (arrows, β -III tubulin; pink). Scale bar, 20 μ m. (B) Representative *en face* confocal images of glioblastoma patient samples processed for PLA using anti-neurexin-3 and neuroligin-1 antibodies (positive control). Red, positive PLA signal. Additional PLA controls using glioblastoma patient samples include omission of primary antibodies (C) as well as performing PLA with two antibodies to proteins that do not interact. In this instance, the primary antibodies were directed against neuroligin-1 and TIM3. Scale bar, 50 μ m. (D). Representative images of HGSOC (E) and HPV HNSCC (F) patient samples processed for PLA using anti-neurexin-3 and neuroligin-1 antibodies (red puncta) together with immunofluorescence for β -III tubulin (green). Arrows point to areas of PLA in close proximity to immunofluorescent nerves. Scale bar, 50 μ m. (G) Snapshot of MEA recording from a case of HGSOC. (H-J) Representative photomicrographs of hematoxylin and eosin stained sections of cases of HGSOCs that were utilized for MEA analysis. Dotted areas denote tumor. Scale bar, 100 μ m. (K-M) Average MEA activity from normal ovary and benign gynecologic tumors. Signals from all electrodes and all tissue slices were pooled, averaged and compared. Statistical significance was determined by one-way ANOVA. Standard deviation, error bars. ns, not significant. (N-P) Representative photomicrographs of hematoxylin and eosin stained sections of cases of HNSCCs that were utilized for MEA analysis. Dotted areas denote tumor. Scale bar, 100 μ m.

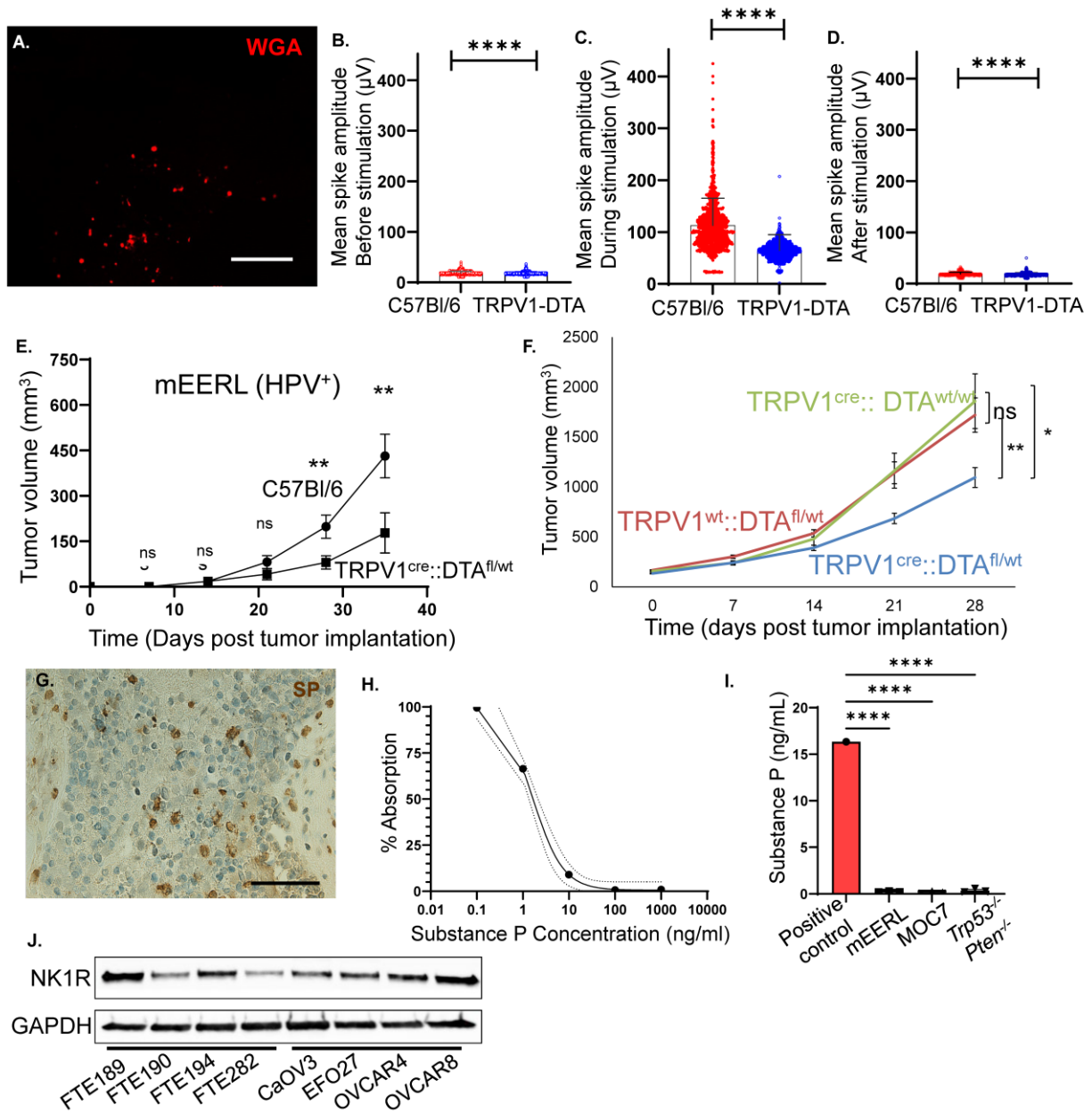


Fig. S3. Intra-tumoral nerves and Substance P. (A) *En face* confocal image of the ipsilateral trigeminal ganglia from a mouse with an oral mEERL (HPV⁺) tumor that was intra-tumorally injected with fluorophore (red) conjugated WGA nerve tracer (Scale bar, 200 μ m). MEA analysis of mEERL tumors (HPV⁺ HNSCC, B-D) subcutaneously implanted in either C57Bl/6 or TRPV1^{cre::DTA^{fl/wt}} (TRPV1-DTA) animals. Signals from all electrodes and all tissue slices were pooled, averaged and compared. Statistical analysis by one-way ANOVA. Standard deviation, error bars. ****, $p < 0.0001$. (E) Tumor growth curve of mEERL tumors orthotopically implanted into C57Bl/6 (circles) or TRPV1^{cre::DTA^{fl/wt}} mice (squares) ($n=10$ mice/group). Statistical analysis by multiple students t-test. **, $p < 0.001$, ns, not significant. (F) Tumor growth curves of the indicated mice implanted in the hindlimb with mEERL-EphrinB1 tumors. Statistical analysis by multiple students t-test. *, $p < 0.05$, **, $p < 0.01$. (G) Photomicrograph of HGSOC patient sample IHC stained for Substance P (brown). $N=4$ cases stained. Scale bar, 100 μ m. (H) Standard curve for Substance P in ELISA. (I) Extrapolation of Substance P concentration in the conditioned media from the indicated cell lines based on the Substance P standard curve by ELISA. Statistical analysis by one-way ANOVA with multiple comparisons. ****, $p < 0.0001$. Positive control, recombinant Substance P. (J) Western blot analysis of whole cell lysates from the indicated human HGSOC as well as control fallopian tube secretory epithelial cell (FTSEC) cell lines for NK1R. GAPDH included as a loading control.

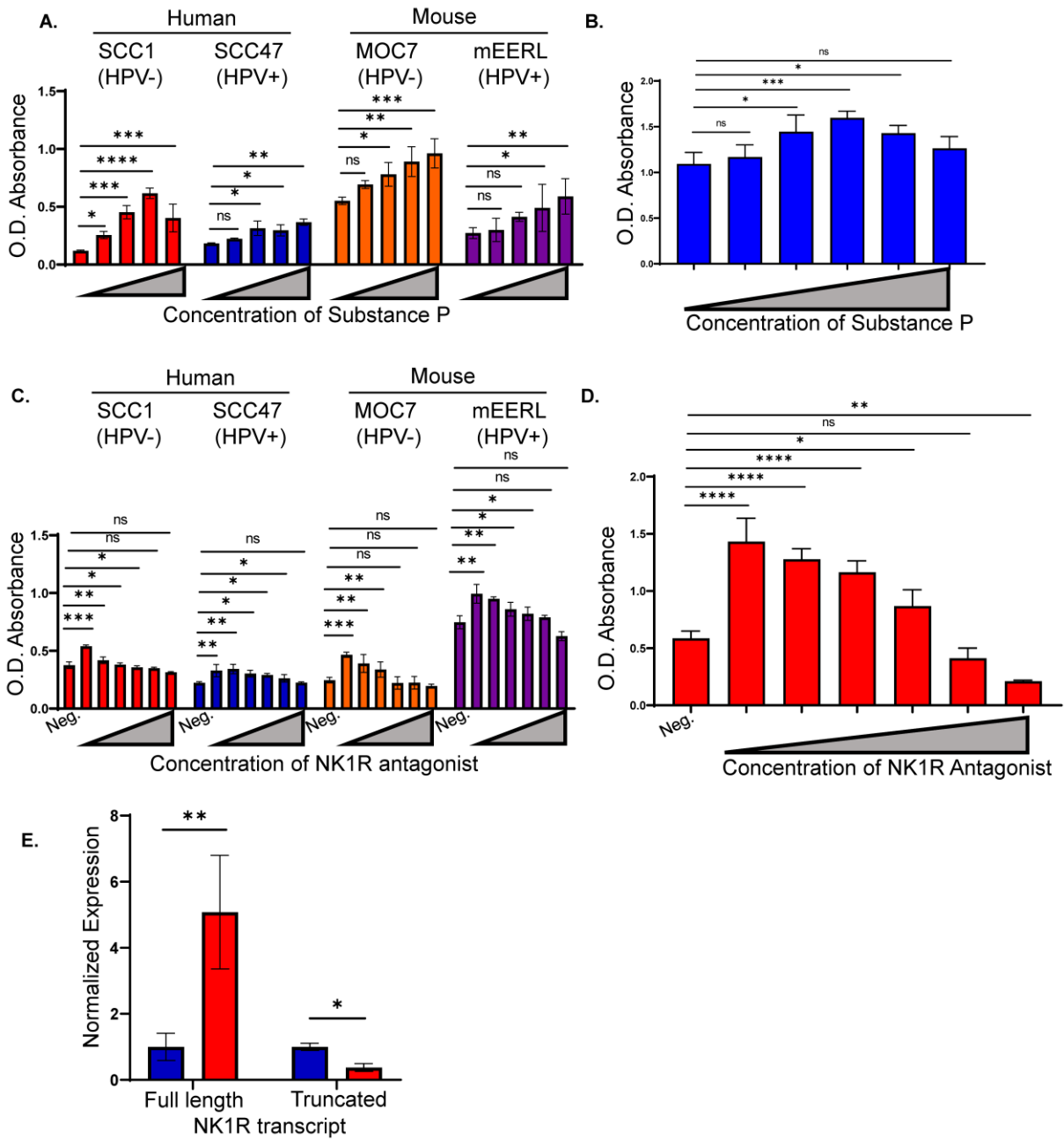


Fig. S4. Substance P promotes proliferation of tumor cells. Cellular proliferation of the indicated HNSCC (A) or HGSOC (B) cell lines in response to increasing concentrations of Substance P for 48 hours; inclusion of an anti-NK1R antagonist attenuates this effect (C, HNSCC; D, HGSOC). Statistical test by one-way ANOVA with post-hoc Tukey test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant. (E) Quantitative RT-PCR analysis for full length and truncated NK1R expression in SCC1 (HPV-, red) and SCC47 (HPV+, blue) cells. Statistical analysis by multiple student's t-tests using Holm-Sidak method. *, $p < 0.05$; **, $p < 0.01$. There were three biological replicates each with six technical replicates; each showing similar results.

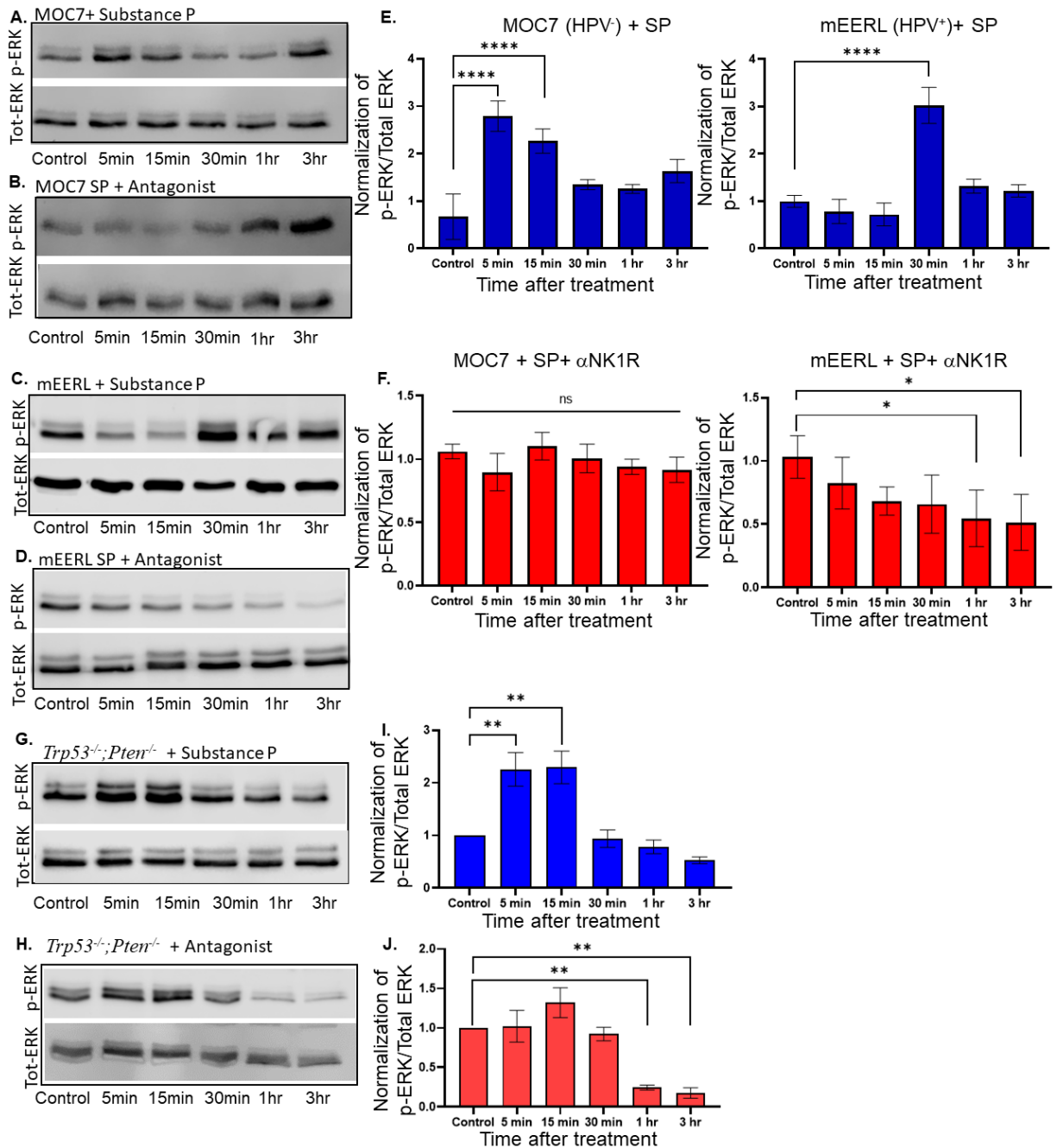


Fig. S5. Substance P-mediated signaling. Western blot analysis for p-ERK and total-ERK of MOC7 (HPV⁻) or mEERL (HPV⁺) cells treated with SP alone (A, C respectively) or in the presence of the NK1R antagonist (B, D respectively) for indicated time points. Densitometric quantification (p-ERK/total ERK) for MOC7 (HPV⁻) and mEERL (HPV⁺) cells treated with SP alone (E) or in the presence of NK1R antagonist (F). Statistical analysis by one-way ANOVA with multiple comparisons. *, p < 0.05, ****, p < 0.0001; all other comparisons were not significant. Western blot analysis for p-ERK and total-ERK of *Trp53^{-/-};Pten^{-/-}* cells treated with Substance P alone (G) or in the presence of the NK1R antagonist (H) for the indicated times. Densitometric quantification (p-ERK/total ERK) of cells treated with Substance P alone (I) or in the presence of NK1R antagonist (J). Statistical analysis by one-way ANOVA with multiple comparisons. *, p < 0.05, **, p < 0.01; all other comparisons were not significant.

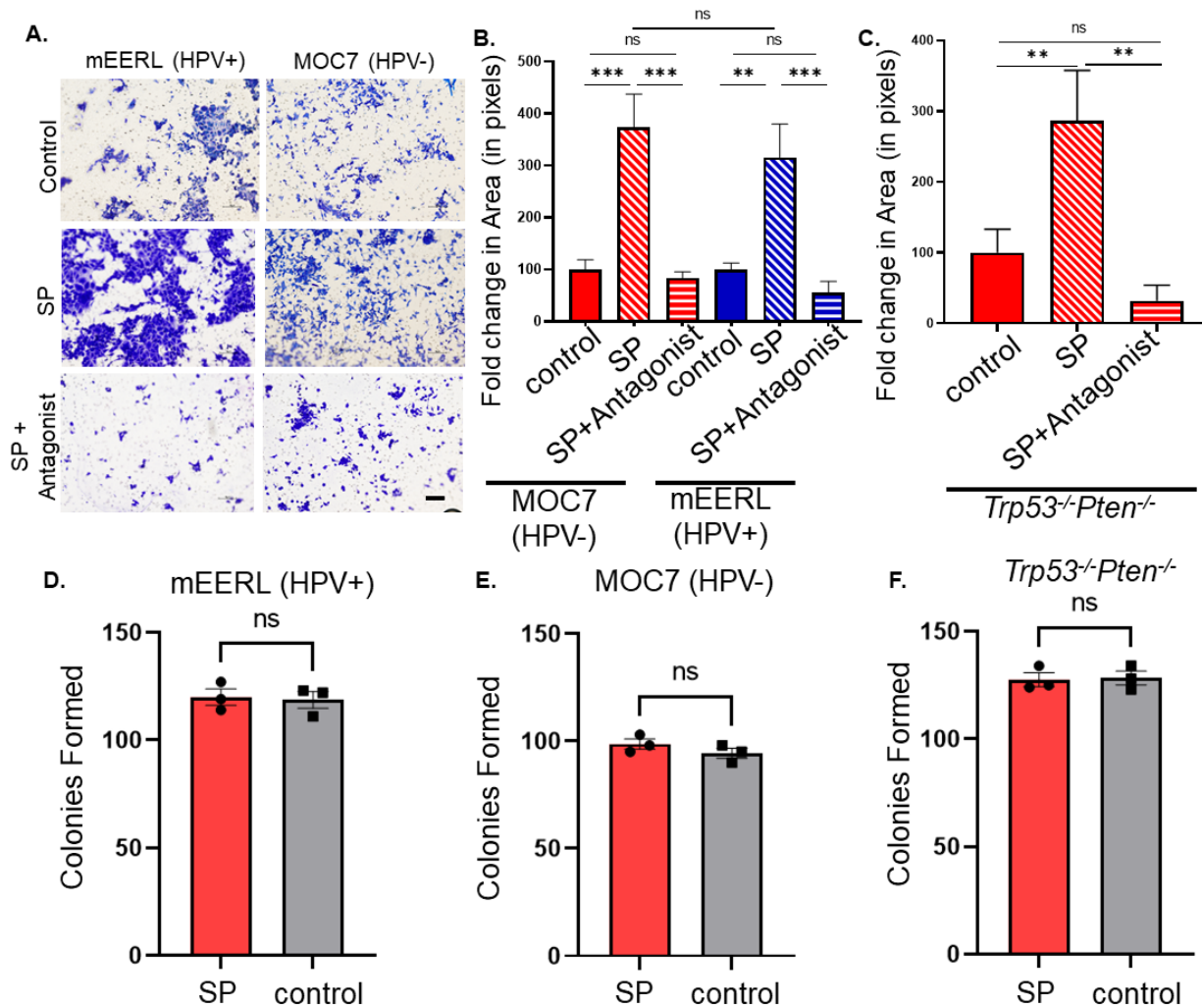


Fig. S6. Substance P-mediated cellular migration. (A) Photomicrographs of transwell migration assays of the indicated cells alone (control), treated with Substance P (SP) or SP with the NK1R antagonist (SP+Antagonist). Scale bar, 100µm. (B) Quantification of HNSCC cellular migration assay; MOC7 (HPV-, red) and mEERL (HPV+, blue). (C) Quantification of cellular migration assay of HGSOC cells (*Trp53*^{-/-}; *Pten*^{-/-} cells) under the indicated conditions. N=3 biological replicates, n=4 technical replicates. Statistical test by one-way ANOVA with post-hoc Tukey test. **, p<0.01; ***, p<0.001; ns, not significant. Quantification of clonogenic assay for mEERL (HPV+) (D), MOC7 (HPV-) (E) and *Trp53*^{-/-}; *Pten*^{-/-} (F) cells treated with Substance P (100nM) or vehicle; n=3 technical replicates; n=2 biological replicates. Statistical analysis by student's t-test; ns, not significant.

Gene	Survival Ovarian cancer		Fold change
	Low	High	
<i>KCNT1</i>	1713.00	760.00	2.25
<i>RAMP3</i>	1009.00	480.00	2.10
<i>GRID2</i>	1657.00	803.00	2.06
<i>AVPR2</i>	1413.00	785.00	1.80
<i>ETV1</i>	1342.00	747.00	1.80
<i>NEFH</i>	1434.00	918.00	1.56
<i>NPY1R</i>	1268.00	848.00	1.50
<i>SCN7A</i>	1342.00	916.00	1.47
<i>PIEZO2</i>	1094.00	758.00	1.44
<i>SLC6A1</i>	1306.00	906.00	1.44
<i>NTF3</i>	1189.00	836.00	1.42
<i>LEP</i>	1249.00	896.00	1.39
<i>SCG2</i>	1249.00	896.00	1.39
<i>C3AR1</i>	1254.00	928.00	1.35
<i>IGF1</i>	1165.00	864.00	1.35
<i>NMU</i>	1208.00	930.00	1.30
<i>BRN3A</i>	1265.00	981.00	1.29
<i>RELN</i>	1292.00	1009.00	1.28
<i>ITGAM</i>	1223.00	968.00	1.26
<i>ADCYAP1</i>	1234.00	987.00	1.25
<i>TACR2</i>	1160.00	928.00	1.25
<i>LEPR</i>	1235.00	995.00	1.24
<i>ADIPOR2</i>	1218.00	1002.00	1.22
<i>CACNA1C</i>	1209.00	1011.00	1.20
<i>CRHR1</i>	1209.00	1011.00	1.20
<i>KCNMB2</i>	1209.00	1012.00	1.19
<i>PTH1R</i>	1122.00	942.00	1.19
<i>NF1</i>	1206.00	1015.00	1.19
<i>FLRT3</i>	1175.00	999.00	1.18
<i>IGF2R</i>	1153.00	983.00	1.17
<i>KCNS1</i>	1215.00	1039.00	1.17
<i>GABRG1</i>	1151.00	993.00	1.16
<i>KCNA1</i>	1182.00	1029.00	1.15
<i>IGF2</i>	1180.00	1040.00	1.13
<i>NUCB1</i>	1176.00	1045.00	1.13
<i>AGT</i>	1171.00	1045.00	1.12
<i>TRPV4</i>	1173.00	1047.00	1.12
<i>KCNC3</i>	1152.00	1069.00	1.08
<i>GRID1</i>	1134.00	1087.00	1.04
<i>AGTR1</i>	1064.00	1024.00	1.04
<i>CASK</i>	1112.00	1097.00	1.01
<i>NPPA</i>	967.00	1035.00	0.93
<i>KCNN3</i>	1077.00	1227.00	0.88
<i>RLN1</i>	998.00	1144.00	0.87
<i>C1QBP</i>	1038.00	1198.00	0.87
<i>UCN2</i>	1031.00	1190.00	0.87
<i>RXFP1</i>	1024.00	1197.00	0.86
<i>GARS</i>	1006.00	1222.00	0.82
<i>LPAR3</i>	951.00	1182.00	0.80
<i>NPY2R</i>	1079.00	1378.00	0.78
<i>NUCB2</i>	981.00	1257.00	0.78
<i>FOXA2</i>	972.00	1248.00	0.78
<i>KISS1</i>	957.00	1229.00	0.78
<i>APLN</i>	924.00	1284.00	0.72
<i>KCNS3</i>	733.00	1238.00	0.59
<i>CD55</i>	842.00	1454.00	0.58

Table S1. List of all the neuron-associated genes expressed in HGSOc and their impact on patient survival.

Gene	Survival HNSCC		Fold change
	Low	High	
MPZ	978.00	516.00	1.90
GAL	1026.00	655.00	1.57
PMCH	1306.00	840.00	1.55
PLCB3	1086.00	786.00	1.38
SLC1A3	1042.00	764.00	1.36
FOXA2	1036.00	797.00	1.30
LDHB	1023.00	812.00	1.26
NUTF2	1014.00	817.00	1.24
GAST	1011.00	820.00	1.23
GATA2	992.00	813.00	1.22
MNX1	1000.00	824.00	1.21
KCNF1	1000.00	833.00	1.20
LPAR3	995.00	832.00	1.20
GARS	968.00	819.00	1.18
SCN2A	982.00	831.00	1.18
RYR2	989.00	843.00	1.17
CD55	986.00	847.00	1.16
KCNMB4	981.00	850.00	1.15
CHGB	978.00	854.00	1.15
TRPM8	977.00	861.00	1.13
ADM	970.00	860.00	1.13
NPBWR1	927.00	823.00	1.13
KCNT1	1008.00	907.00	1.11
RET	970.00	875.00	1.11
FGFR4	952.00	877.00	1.09
SCN5A	953.00	878.00	1.09
ENO2	951.00	880.00	1.08
CHRNA1	944.00	889.00	1.06
GAP43	941.00	889.00	1.06
NPFF	941.00	892.00	1.05
HAPLN4	939.00	895.00	1.05
SCN9A	1072.00	1038.00	1.03
PNOC	929.00	904.00	1.03
GNRH1	926.00	907.00	1.02
AGT	943.00	932.00	1.01
IGF1R	917.00	917.00	1.00
ATF2	892.00	898.00	0.99
GABRA3	908.00	915.00	0.99
CBLN2	909.00	922.00	0.99
SCN4A	899.00	915.00	0.98
AVPR2	908.00	926.00	0.98
NPB	901.00	922.00	0.98
GFRA2	868.00	901.00	0.96
CACNA1D	885.00	931.00	0.95
KCNC3	891.00	941.00	0.95
KCNS1	840.00	888.00	0.95
UCN2	891.00	942.00	0.95
TRPV1	889.00	945.00	0.94
GNRHR	882.00	953.00	0.93
PENK	851.00	925.00	0.92
GHRL	874.00	956.00	0.91
GIPR	866.00	952.00	0.91
SPRR1A	919.00	1021.00	0.90
S100B	855.00	960.00	0.89
CACNA1A	861.00	971.00	0.89
NTRK1	861.00	972.00	0.89
UTS2	805.00	909.00	0.89
POU4F1	867.00	996.00	0.87
AVIL	850.00	983.00	0.86
EDNRB	848.00	982.00	0.86
NPR1	843.00	980.00	0.86
EOMES	914.00	1067.00	0.86
MOXD1	872.00	1018.00	0.86
TRPV4	887.00	1036.00	0.86
NTRK2	843.00	991.00	0.85
TACR1	840.00	991.00	0.85
SH2D3C	840.00	996.00	0.84
RAMP2	807.00	965.00	0.84
RAMP3	828.00	992.00	0.83
CBLN3	830.00	996.00	0.83
PTPRC	827.00	1005.00	0.82
DBH	847.00	1039.00	0.82
NTF3	819.00	1010.00	0.81
KCNN1	678.00	846.00	0.80
NEFH	751.00	951.00	0.79
VIPR1	808.00	1131.00	0.71
KCNN3	775.00	1108.00	0.70
NOTCH1	751.00	1100.00	0.68
SERPINF1	794.00	1280.00	0.62

Table S2. List of all the neuron-associated genes expressed in HNSCC and their impact on patient survival.