Supplementary Methods

Retrospective patient studies

Information collected from electronic medical records included age, gender, body mass index (BMI), stage of disease, tumor location, presence/absence of perineural invasion, pre-operative pain intensity using a verbal numeric rating scale (VNRS; 0 = no pain, 10 = worse pain ever) at the time of anesthesia assessment, history of chronic pain (pain lasting > 3 months), and preoperative analgesic pharmacotherapy use (NSAIDs, gabapentinoids, topical lidocaine lozenge, and opioids). Only patients undergoing initial cancer surgery were included in the analyses.

Cancer cells

We utilized the human HNSCC cell line Fadu, consisting of primary hypopharyngeal squamous cell carcinoma (SCC) tumor cells harvested from a 56-year old male patient. Fadu cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium pyruvate, nonessential amino acids, and a two-fold vitamin solution (Life Technologies, Inc., Grand Island, NY). Adherent monolayer cultures were maintained on plastic plates and incubated at 37°C in 5% CO_2 and 95% air. The cultures were free of Mycoplasma species. Cells were used in co-culture studies 10-20 passages after initial seeding. When prepared for co-culture, the medium was poured off and rinsed with phosphate-buffered saline (PBS). Trypsin was added for 5 min to detach cells from the flask. Enzymatic action was stopped with a sufficient volume of medium. Cells were then pelleted, re-suspended and then counted by TC20 automated cell counter (Bio-Rad Laboratories, Hercules, CA) and re-suspended in medium for implantation at a concentration of 1×10^5 cells/2mL. For conditioned

media studies, Fadu cells were seeded in 10 mL fresh media in a T75 culture flask, and the conditioned media was collected 24 h after passage.

Co-culture procedure

Human DRG culture procedure

Briefly, each donor was undergoing surgical treatment that necessitated ligation of spinal nerve roots to facilitate tumor resection or spinal reconstruction. Spinal roots were ligated proximal to the DRG, spinal root sharply cut both proximal and distal to the DRG, and excised DRG transferred immediately into cold (~4°C) and sterile balanced salt solution. DRG were transported to the laboratory on ice in a sterile, sealed 50-ml centrifuge tube. Upon arrival to the laboratory, each ganglion was carefully dissected from the surrounding connective tissues and cut into several ~1–2-mm pieces and cells dissociated for electrophysiology recording. DRG pieces were placed in a Petri-dish containing trypsin (0.0625 mg/mL, Hyclone, Logan, UT), type IA collagenase (1 mg/mL, Sigma-Aldrich, St. Louis, MO), and DNase (0.0625 mg/mL, Sigma-Aldrich, St. Louis, MO) in DMEM/F12 and shaken for 20 min in a heated (37°C) bath. After each 20 min session, the digestion solution was collected, placed into a blocking solution, and replaced with fresh digestion solution. This process was repeated until the sections were adequately digested. The collected digestion solution and blocking solution was centrifuged at 23°C and 180 RCF for 5 min. The supernatant was removed, and the cell pellet re-suspended with 1 ml of culture media (DMEM/F12 with 10% horse serum). An additional 2-3 ml of culture media was added, and then the suspension filtered through a 100-micron cell strainer. The remaining suspension was centrifuged at 23°C and 180 RCF for 5 min. The supernatant was removed, and the cell pellet re-suspended with 600 µL culture media.

Rat DRG culture procedure

Rats were deeply anesthetized with SomnaSol (pentobarbital 390 mg/mL and phenytoin 50 mg/mL) and perfused with chilled saline on ice. Both pairs of the L4 and L5 DRG were excised and placed in a culture dish containing trypsin (0.0625 mg/mL, Hyclone, Logan, UT) and type IA collagenase (1 mg/mL, Sigma-Aldrich, St. Louis, MO) in DMEM. The dish was shaken in a heated chamber for 50 min at 37°C. The cells were then washed and mechanically dispersed. The suspension was filtered through a 70 μm cell strainer and centrifuged at 180 RCF for 5-7 min at 23°C. The supernatant was removed, and the cell pellet resuspended in 600 μL culture media.

Enzyme-Linked Immunosorbent Assay (ELISA)

Human DRG

Using a pre-coated 96-well microplate, 100 μL of Assay Diluent RD1X was added to each well, followed by 50 μL of standard, control, or sample (cell culture supernatants). The plate was covered and incubated for 2 hours at room temperature on a horizontal orbital shaker. Each well was aspirated and washed three times using 400 μL wash buffer. Each well was then filled with the appropriate conjugate (200 μL of Human IL-6 Conjugate). The plate was then covered and incubated for 1 h at room temperature on a horizontal orbital shaker. The previous aspiration/wash step was then repeated. After 200 μL of Substrate Solution was added to each well, the plate was incubated for 20 minutes at room temperature while protected from light. Finally, 50 μL of Stop Solution was added to each well and the optical density of each well was determined within 30 minutes using a SpectraMax plus 384 Microplate Reader (Molecular Devices, San Jose, CA) set to 450 nm. IL-6 values were expressed as ng/mL. It should be noted that in analyzing media samples, we are unable to calculate total protein values for each sample,

and therefore cannot normalize to total protein in these assays. We cannot exclude the possibility that there may be a high degree of variability among samples due to differences in the total number of cells in each well.

Rat DRG

Rat IL-6 levels were assessed using the Rat IL-6 Quantikine ELISA Kit (Cat no: R6000B, R&D Systems, USA). Briefly, using a pre-coated 96-well microplate, 50 µL of Assay Diluent RD1-54 was added to each well, followed by 50 µL of standard, control, or sample (cell culture supernatants). The plate was covered and incubated for 2 hours at room temperature on a horizontal orbital shaker. Each well was aspirated and washed five times using 400 µL wash buffer. Each well was then filled with the appropriate conjugate (100 µL of Rat IL-6 Conjugate). The plate was then covered and incubated for 2 hours at room temperature on a horizontal orbital shaker. The previous aspiration/wash step was then repeated. Then 100 µL of Substrate Solution was added to each well, the plate was incubated for 30 minutes at room temperature while protected from light. Finally, 100 µL of Stop Solution was added to each well and the optical density of each well was determined within 30 minutes using a SpectraMax plus 384 Microplate Reader (Molecular Devices, San Jose, CA) set to 450 and 540 nm. IL-6 values were expressed as ng/mL. As noted above, in analyzing media samples, we are unable to calculate total protein values for each sample, and therefore cannot normalize to total protein in these assays. We cannot exclude the possibility that there may be a high degree of variability among samples due to differences in the total number of cells in each well.

Chemiluminescence Assay

Human Neuro Discovery Antibody Array C2 was purchased from Ray Biotech (Norcross, GA, USA). The Human array was designed to detect 30 human cytokines that play important roles in inflammation, innate immunity, apoptosis, angiogenesis, cell growth and differentiation.

We also tested media samples using Rat Neuro Discovery Antibody Array C1 from Ray Biotech (Norcross, GA, USA). This assay detects 19 rat cytokines that play important roles in inflammation, innate immunity, apoptosis, angiogenesis, cell growth and differentiation.

Electrophysiology

Glass coverslips were lifted and were transferred to a recording chamber and perfused at 2 mL/min with oxygenated (95% O_2 + 5% CO_2) extracellular solution containing 117 mM NaCl, 3.6 mM KCl, 1.2 mM $NaH_2PO_4 \cdot H_2O$, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$ and 11 mM glucose adjusted to pH 7.4 with NaOH. Glass micropipettes (6–8 MΩ) were filled with an internal solution of 135 mM K-gluconate, 5 mM KCl, 5 mM Mg-ATP, 0.5 mM Na_2GTP, 5 mM HEPES, 2 mM $MgCl_2$, 5 mM EGTA, and 0.5 mM $CaCl_2$ adjusted to pH 7.4 with KOH and an osmolarity of 290–300 mOsm. Series resistance (Rs) was compensated to above 70%. All recordings were made at room temperature.

Immunocytochemistry

DRG neurons, Fadu cancer cells, or both in Co-culture were seeded as described earlier on a poly-L-lysine-coated glass bottom 6-well plate (#1.5 cover glass, Cellvis, Mountainview, CA, USA) without coverslips. After 24 h, culture media was washed with 1X PBS, and cells fixed in 4% paraformaldehyde for 30 min at 4C. The plate was then washed with 1X PBS, and tissue blocked for 1 h at room temperature using 5% normal donkey serum and 0.2% Triton X-100 in 1X PBS. The blocking solution was then removed and the tissues incubated overnight at 4°C in

1% normal donkey serum and 0.2% Triton X-100 in PBS containing primary antibodies against the following targets: neuronal beta III tubulin (rabbit anti-rat, 1:500, ab229590 abcam, Boston, MA), cytokeratin (mouse anti-human, 1:500, C2562 Sigma-Aldrich, Inc., St. Louis, MO). After the plate was washed using 1X PBS, tissues were incubated in 1% normal donkey serum and 0.2% Triton X-100 in PBS containing 1:500 Cy5-conjugated secondary antibodies (donkey anti-mouse, 715-175-151 Jackson ImmunoResearch Inc., West Grove, PA) and 1:1000 FITC-conjugated secondary antibodies (goat anti-rabbit, ab6717 abcam, Boston, MA) overnight at 4°C. The plate was washed with PBS and 2 mL of PBS was added to each well before imaging. Culture wells were viewed with use of a fluorescence microscope (A1 HD25; Nikon, Melville, NY). For a given experiment, all images were taken using identical acquisition parameters.

Statistical analysis

A multivariate logistic regression model was used to evaluate the effect of covariates on oral cancer pain. All important covariates were included in the full model for model selection. A backward model selection method was used. The final model includes the covariates with a p-value less than 0.20.

For ELISA of human IL-6 in media from human DRG studies, expression levels in ng/mL were compared among DRG only culture, Fadu cancer cell culture, and DRG Co-culture with Fadu cancer cells using one-way ANOVA. Bonferroni post-hoc tests were used to identify significant differences between the groups. For ELISA of rat IL-6 in media from rat DRG studies, expression levels in ng/mL were compared among the different sex (male vs. female), age (older adult vs. younger adult), and treatment (media only vs. co-culture) groups using a three-way ANOVA and Bonferroni post-hoc tests. Fadu only media samples collected at the same time as media only vs. co-culture samples were analyzed simultaneously but were not

included in further analyses since they only contained human cancer cells. Mean rat IL-6 expression from these samples was found to be 14.82±2.21 ng/mL.

For the human and rat chemiluminescence assays, cytokine, chemokine, and growth factor levels in co-culture media were normalized to media only and Fadu only to compensate for potential differences in positive control intensity readings between experiments. For some experiments, Fadu only media served as a control for multiple rat DRG experiments run at the same time. Cytokines were considered to demonstrate significant upregulation if there was ≥2-fold mean increase relative to media only and/or Fadu only. For human assays, cytokine levels in Media Only, Fadu only, and Co-culture media are also presented without normalization as median optical intensity units and were compared using non-parametric Kruskal-Wallis tests.

Current thresholds (rheobase, in pA), resting membrane potential (RMP, in mV), and action potential (AP) characteristics at rheobase were compared between DRG neurons in co-culture with Fadu cancer cells and those incubated in media only using independent *t*-tests. For co-cultured neurons from young adult males, older adult males, and older adult females, rheobase, resting membrane potential (RMP), and AP characteristics were also compared between neurons with spontaneous activity (SA) and those without SA using independent *t*-tests. For young adult females, fewer than 5 neurons demonstrated SA, and so these comparisons are not shown. Differences in the proportion of neurons demonstrating spontaneous activity (SA) or large (>5mV) oscillations in resting membrane potential (depolarizing spontaneous fluctuations, DSFs) were assessed using non-parametric analyses (Chi-square test). Responses to current stimulation at 1X, 2X and 3X rheobase were compared using repeated measures analysis of variance (ANOVA) with treatment group (media only vs co-culture) as the between-subjects

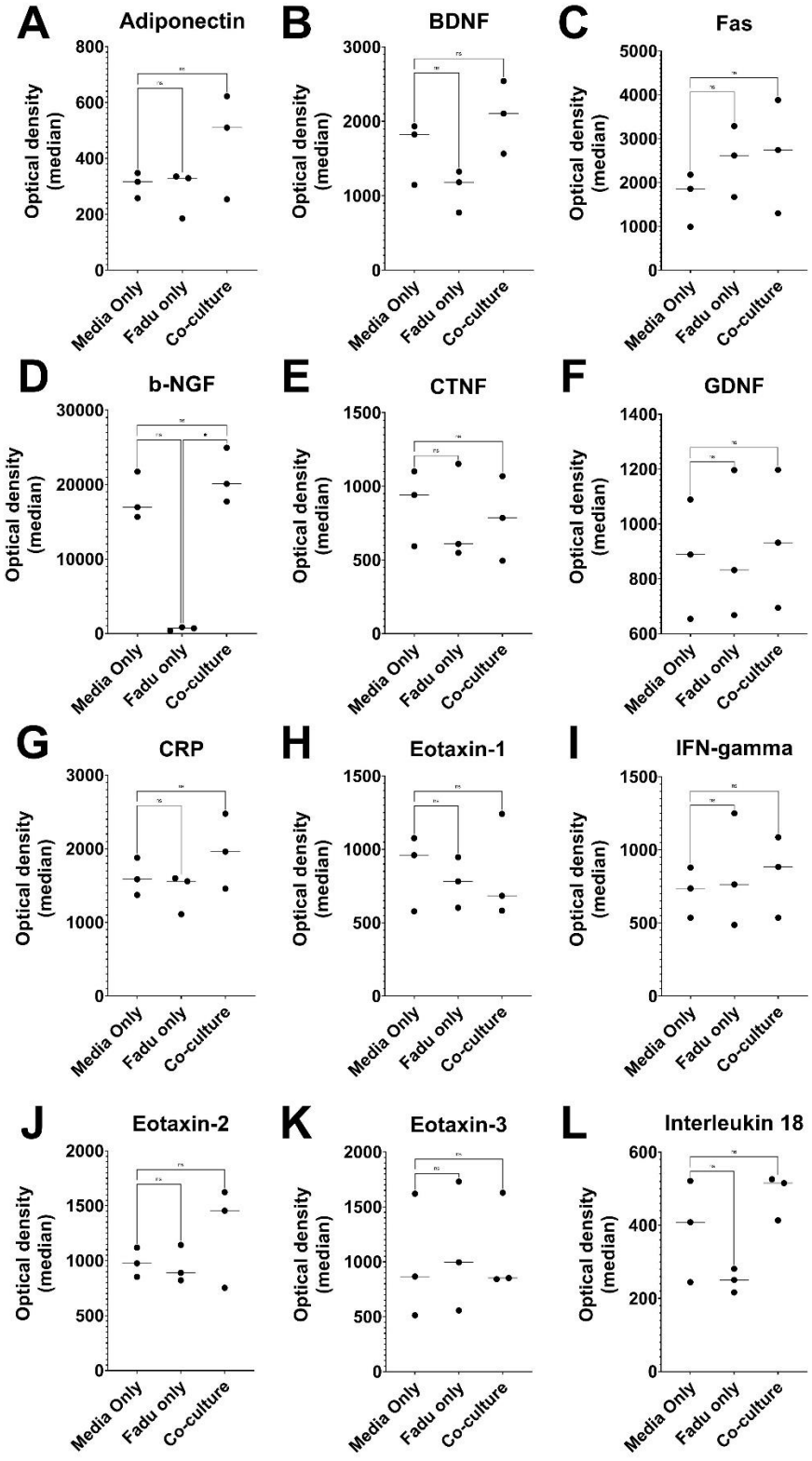
factor and stimulus (1X, 2X, and 3X) as the within-subjects factor. Bonferroni post-hoc tests were used to identify significant differences between the groups.

For Fadu cancer conditioned media experiments, treatment groups (Fadu CCM and control) were compared using independent *t*-tests for current threshold and RMP comparisons and repeated measures analysis of variance (ANOVA) with treatment group as the between-subjects factor and stimulus (1X, 2X, and 3X) as the within-subjects factor followed by Bonferroni post-hoc tests for comparing responses to current stimulation.

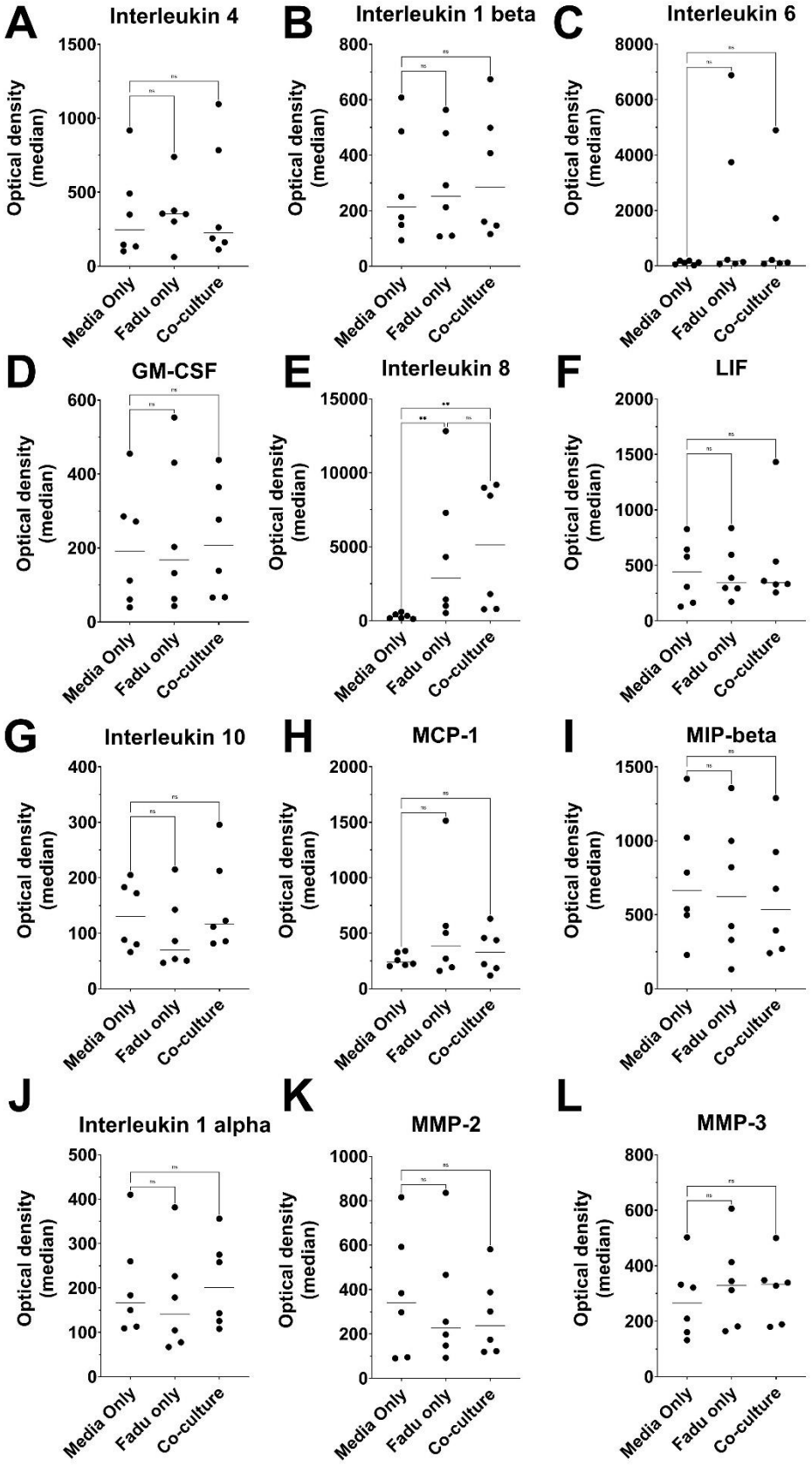
Changes in RMP in response to treatment with rrIL-6 or rhIL-6 were assessed using a dependent *t*-test. For experiments using human IL-6 receptor antagonist Tocilizumab, current thresholds and RMP were compared using one-way ANOVA with four groups (co-culture + vehicle, co-culture + Tocilizumab, media only + Vehicle, media only + Tocilizumab) followed by Bonferroni post-hoc tests.

For all analyses, $p<0.05$ was considered significant.

Supplementary Figures



Supplementary Figure 1. Results from Human Neuro Discovery Antibody Assay Array C2. Using media from co-culture experiments with 3 patient DRGs, median optical intensity was quantified to compare Media Only, Fadu only, and Co-culture conditions. Graphs present data for (A) Adiponectin, (B) Brain-derived neurotrophic factor (BDNF), (C) Fas, (D) Beta Nerve Growth Factor (b-NGF), (E) Ciliary Neurotrophic Factor (CTNF), (F) Glial Cell-Line Derived Neurotrophic Factor (GDNF), (G) C-Reactive Protein (CRP), (H) Eotaxin-1, (I) Interferon Gamma (IFN-G), (J) Eotaxin-2, (K) Eotaxin-3, and (L) Interleukin-18 (IL-18). Statistical analyses (Kruskal-Wallis test) showed median differences for b-NGF ($p=.0250$), with optical intensity for Fadu significantly lower than Co-culture. $*p<.05$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Interleukin 4
Optical density (median)
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Media Only
Fadu only
Co-culture
B
Interleukin 1 beta
Optical density (median)
800
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400
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ns
Media Only
Fadu only
Co-culture
C
Interleukin 6
Optical density (median)
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4000
2000
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Media Only
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Interleukin 8
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Interleukin 1 alpha
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MMP-2
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MMP-3
Optical density (median)
800
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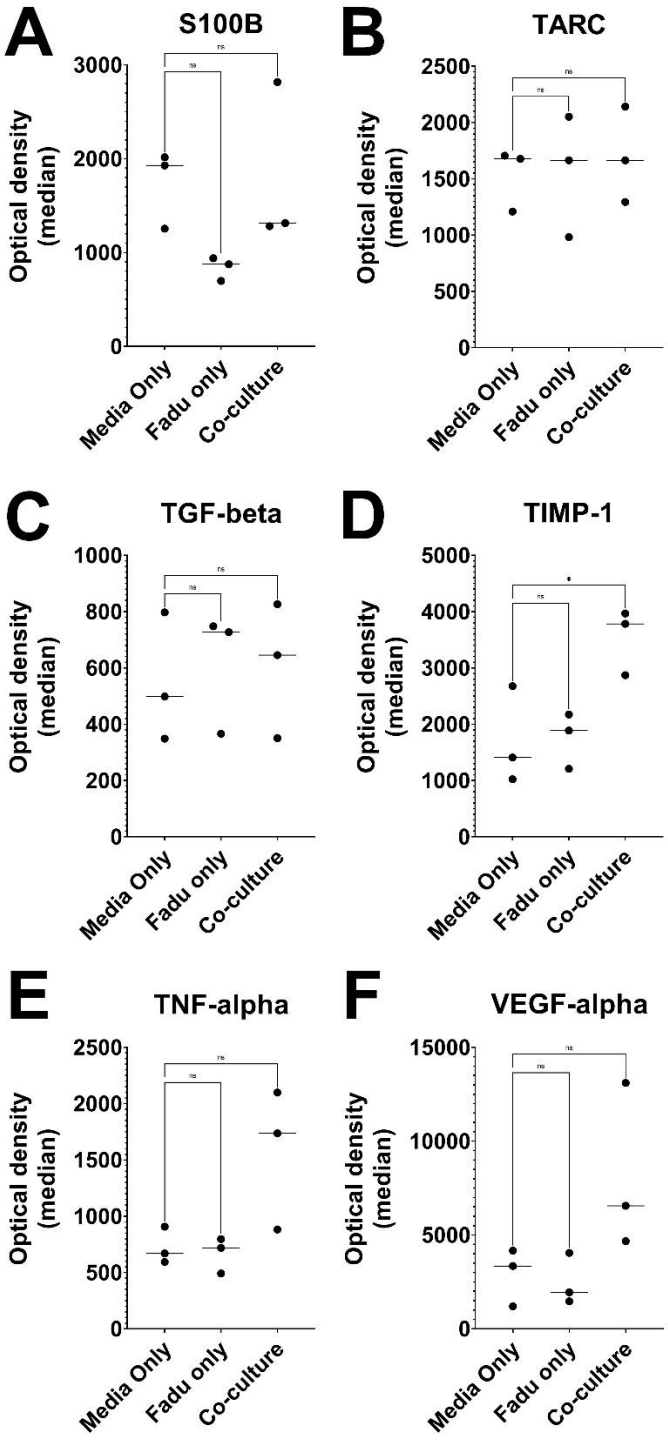
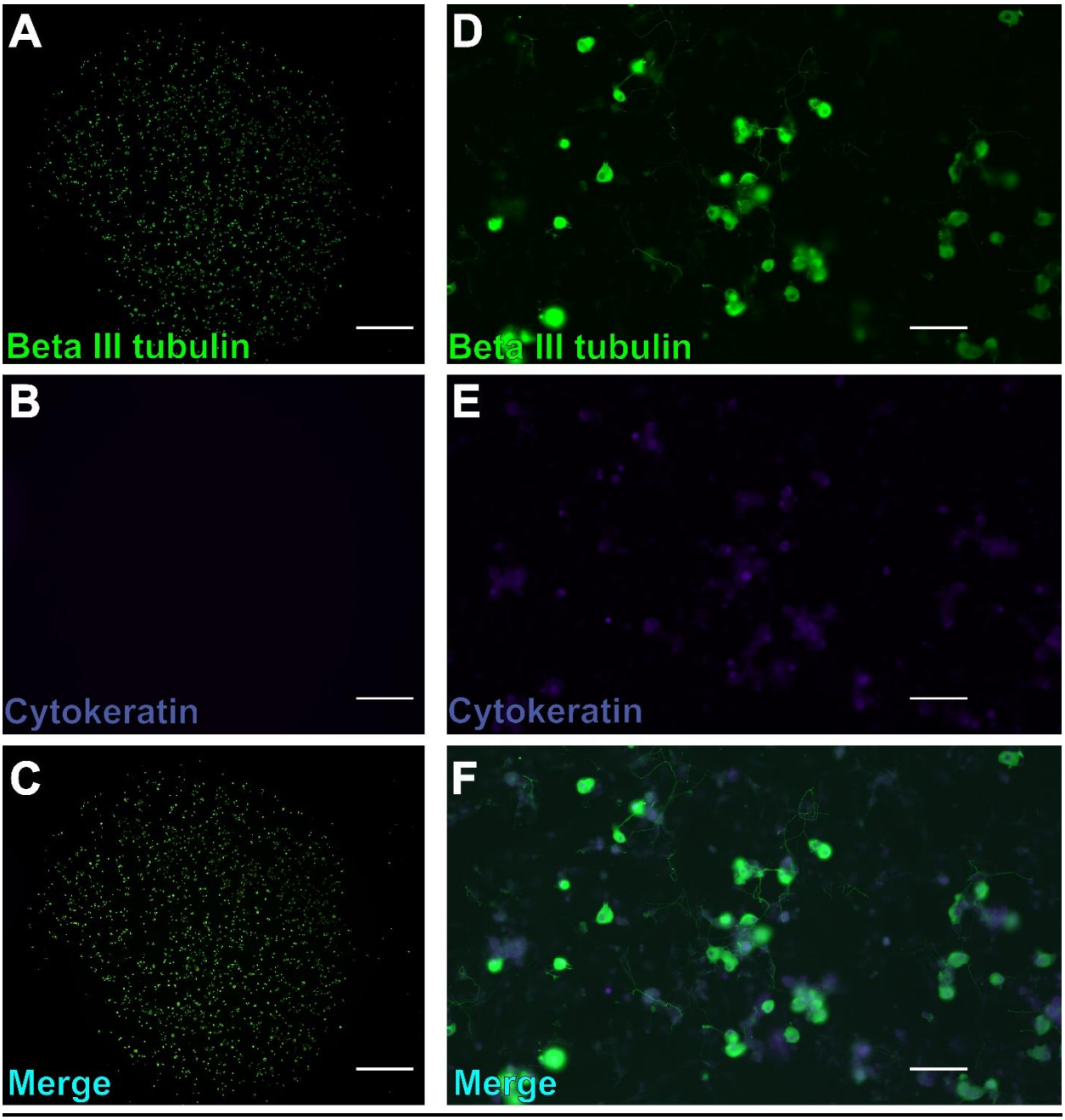
Media Only
Fadu only
Co-culture

Supplementary Figure 2. Results from Human Neuro Discovery Antibody Assay Array C2. Using media from co-culture experiments with 3 patient DRGs, median optical intensity was quantified to compare Media Only, Fadu only, and Co-culture conditions. Graphs present data for (A) Interleukin-4 (IL-4), (B) Interleukin-1 beta (IL-1beta), (C) Interleukin-6 (IL-6), (D) Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), (E) Interleukin-8 (IL-8), (F) Leukemia Inhibitory Factor (LIF), (G) Interleukin-10 (IL-10), (H) Monocyte Chemoattractant Protein-1 (MCP-1), (I) Macrophage Inflammatory Protein-1 Beta (MIP-beta), (J) Interleukin-1 alpha (IL-1alpha), (K) Matrix Metallopeptidase-2 (MMP-2), and (L) Matrix Metallopeptidase-3 (MMP-3). Statistical analyses (Kruskal-Wallis test) showed median differences for IL-10 ($p=.0107$), with optical intensity for Fadu significantly lower than Co-culture, and MIP-beta ($p=.0250$), with optical intensity for Fadu significantly higher than Co-culture. *$p<.05$

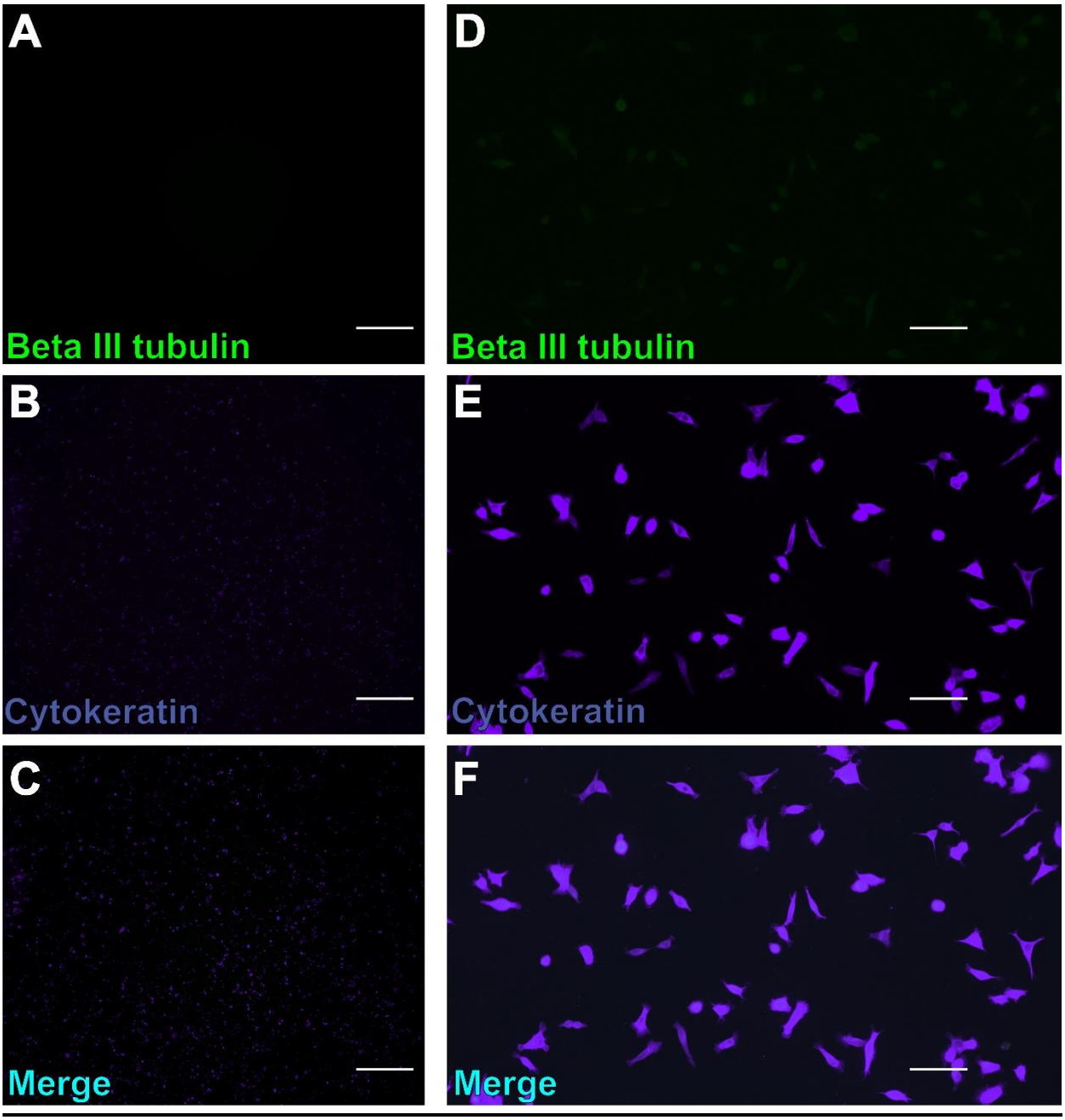


Supplementary Figure 3. Results from Human Neuro Discovery Antibody Assay Array C2. Using media from co-culture experiments with 3 patient DRGs, median optical intensity was quantified to compare Media Only, Fadu only, and Co-culture conditions. Graphs present data

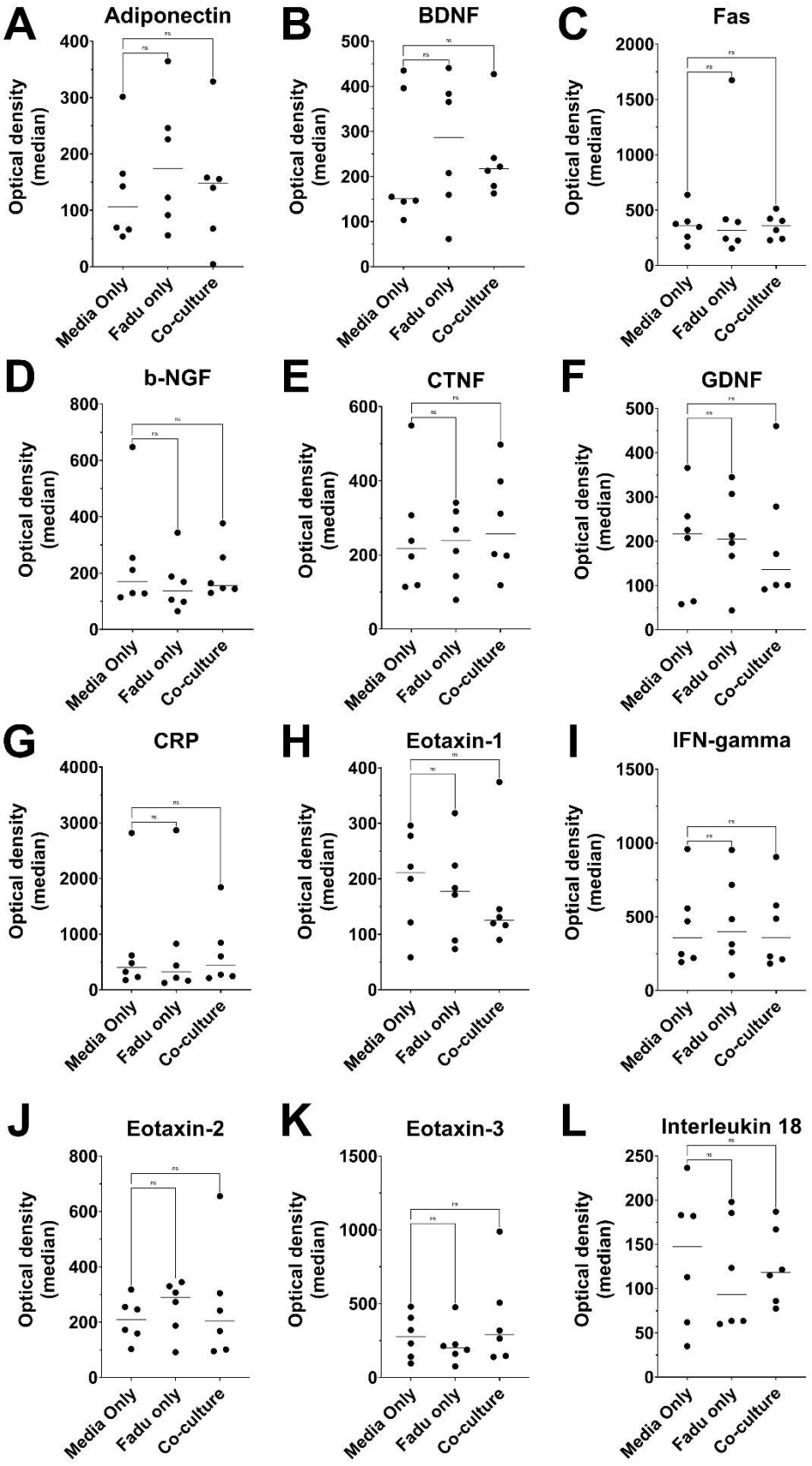
for (A) S100 Calcium Binding Protein B (S100B), (B) Thymus and Activation Regulated Chemokine (TARC), (C) Transforming Growth Factor Beta (TGF-beta), (D) Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1), (E) Tumor Necrosis Factor Alpha (TNF-alpha), and (F) Vascular Endothelial Growth Factor A (VEGF-a).



Supplementary Figure 4. Immunocytochemistry of rat DRG Only culture using 2X objective (A-C) and 20X objective (D-F). Neurons are labeled with neuron-specific beta III tubulin (A,C). Fadu cancer cells (not present in this culture) are labeled with cytokeratin (B,E). A-C Scale bar = 1000 μm; D-F Scale bar = 100 μm.



Supplementary Figure 5. Immunocytochemistry of Fadu Only culture using 2X objective (A-C) and 20X objective (D-F) showing only cytokeratin-positive cells in the area when no caps or cylinders are used during plating. Neurons (not present in this culture) are labeled with neuron-specific beta III tubulin (A,C). Fadu cancer cells are labeled with cytokeratin (B,E). A-C Scale bar = 1000 µm; D-F Scale bar = 100 µm.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BDNF
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CTNF
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Eotaxin-1
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IFN-gamma
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Eotaxin-3
L
Interleukin 18
Optical density (median)
Media Only
Fadu only
Co-culture
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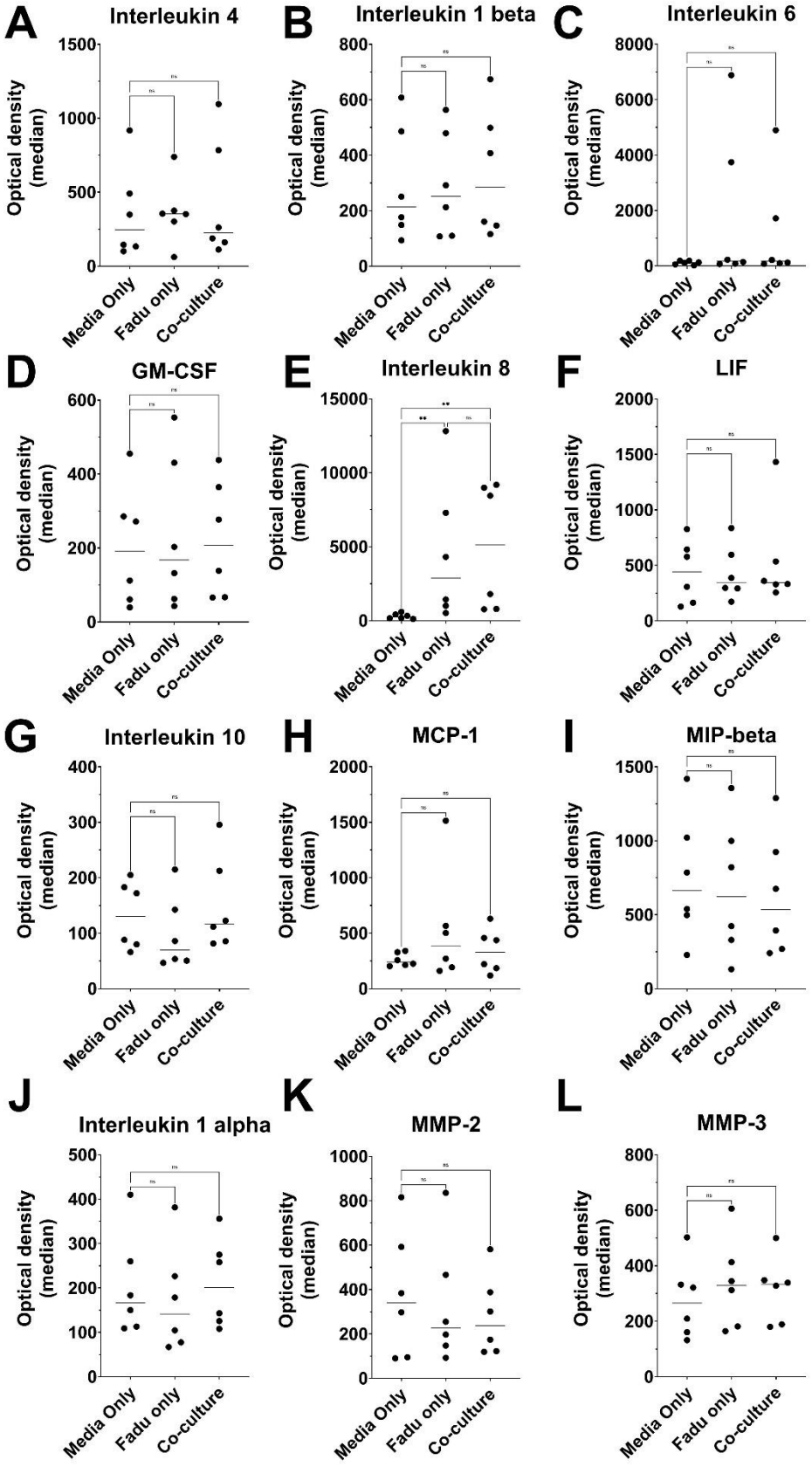
Supplementary Figure 6. Results from Human Neuro Discovery Antibody Assay Array C2. Using media from co-culture experiments with 6 older adult rat DRGs, median optical intensity was quantified to compare Media Only, Fadu only, and Co-culture conditions. Graphs present data for (**A**) Adiponectin, (**B**) Brain-derived neurotrophic factor (BDNF), (**C**) Fas, (**D**) Beta Nerve Growth Factor (b-NGF), (**E**) Ciliary Neurotrophic Factor (CTNF), (**F**) Glial Cell-Line Derived Neurotrophic Factor (GDNF), (**G**) C-Reactive Protein (CRP), (**H**) Eotaxin-1, (**I**) Interferon Gamma (IFN-G), (**J**) Eotaxin-2, (**K**) Eotaxin-3, and (**L**) Interleukin-18 (IL-18).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Interleukin 4
Optical density (median)
1500
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Media Only
Fadu only
Co-culture
B
Interleukin 1 beta
Optical density (median)
800
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400
200
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Media Only
Fadu only
Co-culture
C
Interleukin 6
Optical density (median)
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Media Only
Fadu only
Co-culture
D
GM-CSF
Optical density (median)
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Fadu only
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Interleukin 8
Optical density (median)
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Optical density (median)
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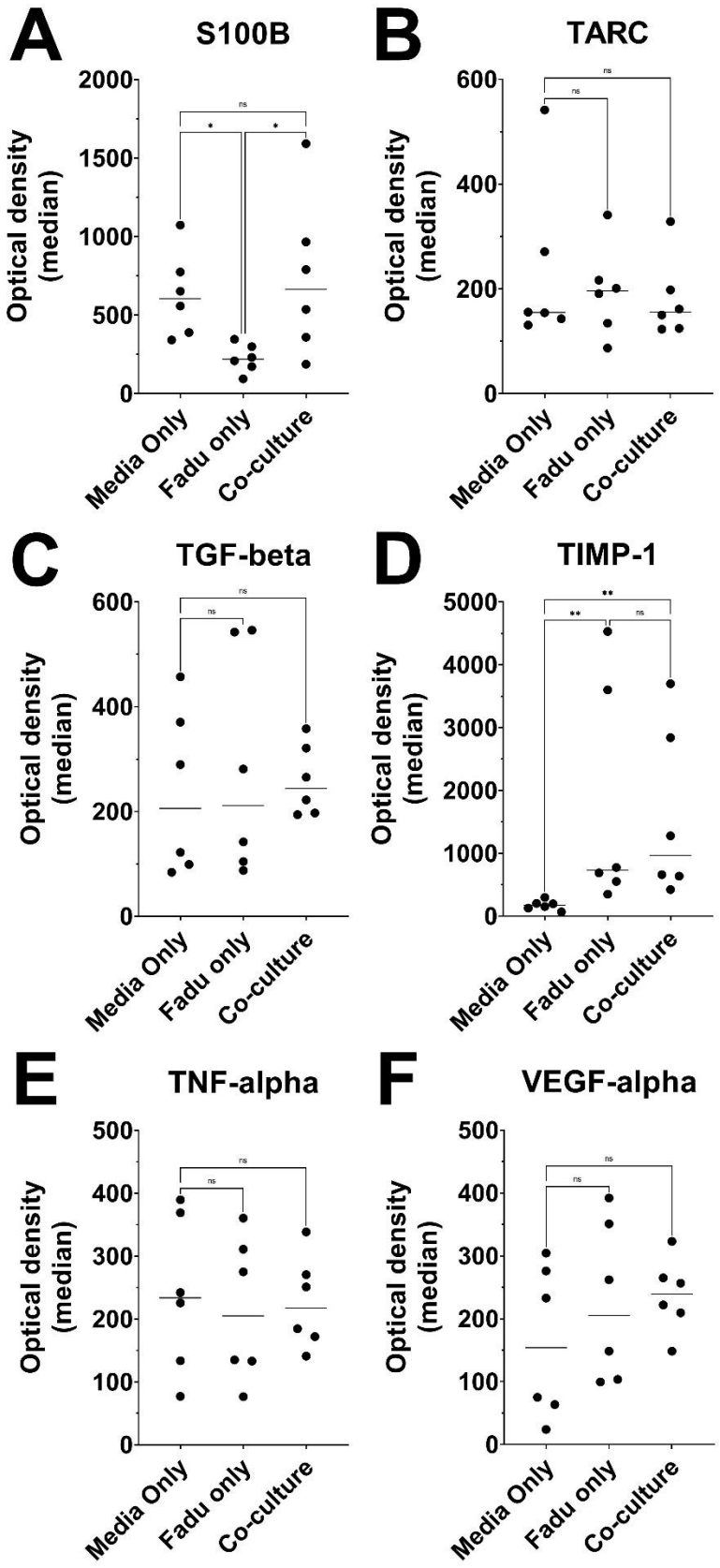
Media Only
Fadu only
Co-culture

Supplementary Figure 7. Results from Human Neuro Discovery Antibody Assay Array C2. Using media from co-culture experiments with 6 older adult rat DRGs, median optical intensity was quantified to compare Media Only, Fadu only, and Co-culture conditions. Graphs present data for (**A**) Interleukin-4 (IL-4), (**B**) Interleukin-1 beta (IL-1beta), (**C**) Interleukin-6 (IL-6), (**D**) Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), (**E**) Interleukin-8 (IL-8), (**F**) Leukemia Inhibitory Factor (LIF), (**G**) Interleukin-10 (IL-10), (**H**) Monocyte Chemoattractant Protein-1 (MCP-1), (**I**) Macrophage Inflammatory Protein-1 Beta (MIP-beta), (**J**) Interleukin-1 alpha (IL-1alpha), (**K**) Matrix Metallopeptidase-2 (MMP-2), and (**L**) Matrix Metallopeptidase-3 (MMP-3). Statistical analyses (Kruskal-Wallis test) showed median differences for IL-8 ($p=.001$), with optical intensity for both Fadu and Co-culture significantly greater than Media Only. **$p<.01$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VEGF-alpha
Optical density (median)
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Media Only
Fadu only
Co-culture

Supplementary Figure 8. Results from Human Neuro Discovery Antibody Assay Array C2. Using media from co-culture experiments with 6 older adult rat DRGs, median optical intensity was quantified to compare Media Only, Fadu only, and Co-culture conditions. Graphs present data for (**A**) S100 Calcium Binding Protein B (S100B), (**B**) Thymus and Activation Regulated Chemokine (TARC), (**C**) Transforming Growth Factor Beta (TGF-beta), (**D**) Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1), (**E**) Tumor Necrosis Factor Alpha (TNF-alpha), and (**F**) Vascular Endothelial Growth Factor A (VEGF-a). Statistical analyses (Kruskal-Wallis test) showed median differences for S100B ($p=.0107$), with optical intensity for Fadu significantly lower than both Media Only and Co-culture, and for TIMP-1 ($p=.0006$), with both Fadu and Co-culture having significantly higher optical intensity than Media Only. *$p<.05$, **$p<.005$

Supplementary Tables

Supplementary Table 1. Human dorsal root ganglion tissue patient demographic information

Donor	Age	DRG[1]	Sex	Ethnicity	Race	Primary Tumor
1	63	T4L,T5L	Male	not Hispanic or Latino	White	Non-small cell lung cancer
2	58	T8R	Male	not Hispanic or Latino	White	Melanoma
3	56	T4L,T5L	Male	not Hispanic or Latino	White	Renal
4	77	T4R	Male	not Hispanic or Latino	White	Prostate
5	67	T8L,T8R	Female	not Hispanic or Latino	White	Adenocarcinoma of the lung
6	81	T8L	Male	not Hispanic or Latino	Black	Unknown
7	53	T7R,T9L	Female	Hispanic or Latino	Other	Malignant Bone Tumor Leiomyosarcoma

DRG = dorsal root ganglion

[1]the level of the thoracic spine and side from which the DRG tissue was harvested

Supplementary Table 2. Demographic data in patients with HPV-negative oral cavity cancers

Variable	Levels	Preop pain=No	Preop pain=Yes	p-value
Age, mean (SD)		59.6(13.87)	57.07(14.31)	.308
Sex, n (%)	Female	25(52.1%)	23(47.9%)	.757
	Male	70(54.7%)	58(45.3%)	
Body mass index, mean (SD)		27.54(5.78)	28.58(6.14)	
Opioid use, n (%)	No	28(51.9%)	26(48.1%)	.706
	Yes	67(54.9%)	55(45.1%)	
Gabapentinoids use, n (%)	No	79(52.7%)	71(47.3%)	.402
	Yes	16(61.5%)	10(38.5%)	
NSAIDs use, n (%)	No	82(58.2%)	59(41.8%)	.025
	Yes	13(37.1%)	22(62.9%)	
NSAID type				.124
Naproxen, n (%)		3(23.1%)	7(31.8%)	
Ibuprofen, n (%)		7(53.8%)	8(36.3%)	
Celecoxib, n (%)		3(23.1%)	0(0%)	
Rofecoxib, n (%)		0(0%)	1(4.6%)	
Meloxican, n (%)		0(0%)	4(18.1%)	
Ketorolac, n (%)		0(0%)	1(4.6%)	
Other		0(0%)	1(4.6%)	
NSAID + Opioids, n (%)	No	71(12.3%)	74(22.1%)	.112
	Yes	10(87.7%)	21(77.9%)	
Pain intensity, mean (SD)	Opioid naïve patients	2.55 (2.56)	0.85 (2.31)	.006
Pathology location	Tongue	49(45.8%)	58(54.2%)	.024
	Floor of mouth	13(81.3%)	3(18.8%)	
	Gingiva	1(20%)	4(80%)	
	Buccal mucosa	7(58.3%)	5(41.7%)	
	Hard palate	2(66.7%)	1(33.3%)	
	RMT	11(64.7%)	6(35.3%)	
	Alveolar ridge	10(83.3%)	2(16.7%)	
	Lip mucosa	1(50%)	1(50%)	
	Other	1(50%)	1(50%)	
Pathological tumor staging	1	22(47.8%)	24(52.2%)	.58
	2	33(53.2%)	29(46.8%)	
	3	12(66.7%)	6(33.3%)	
	4	28(56%)	22(44%)	
Node staging	0	50(49%)	52(51%)	.643
	1	13(61.9%)	8(38.1%)	
	2	4(66.7%)	2(33.3%)	
	3	23(60.5%)	15(39.5%)	
	4	5(55.6%)	4(44.4%)	
Perineural invasion	No	56(49.1%)	58(50.9%)	.079
	Yes	39(62.9%)	23(37.1%)	

Preop: preoperative. HPV: human papilloma virus; NSAIDs: Non-steroidal anti-inflammatory drugs. RMT: retromolar trigone. SD: standard deviation.

Supplementary Table 3. Fold change in optical density for media samples analyzed using Rat Neuro Discovery Antibody Array C1.

	Young Adult Female (n = 2)		Young Adult Male (n = 2)		Older Adult Female (n = 1)		Older Adult Male (n = 1)	
Target	Fold Change vs. Media Only	Fold Change vs. FaDu Only	Fold Change vs. Media Only	Fold Change vs. FaDu Only	Fold Change vs. Media Only	Fold Change vs. FaDu Only	Fold Change vs. Media Only	Fold Change vs. FaDu Only
B-NGF	0.8	0.7	1.0	0.6	1.1	0.9	1.0	1.0
CTNF	0.8	0.6	1.0	0.7	1.1	0.9	1.0	1.0
Eotaxin-1	1.1	1.1	0.6	0.7	1.3	0.9	1.1	1.1
GM-CSF	0.9	1.2	0.4	0.7	0.8	1.4	1.1	1.2
IFN-gamma	0.9	1.4	0.8	0.7	1.0	0.9	0.9	1.1
IL-10	1.1	1.4	0.7	0.7	1.1	0.8	0.9	1.1
IL-1 alpha	1.1	0.8	0.8	0.5	1.2	0.9	0.9	0.9
IL-1 beta	1.9	1.1	0.7	0.6	1.0	0.6	0.9	1.1
IL-4	1.1	1.0	1.7	1.1	1.1	0.5	1.1	1.0
IL-6	1.1	1.1	0.8	0.7	1.4	1.4	1.0	1.1
LIX	1.2	0.9	0.9	0.7	1.0	0.9	1.1	1.2
MCP-1	1.5	1.8	0.9	1.2	1.6	**2.4**	1.7	**2.5**
Neuropilin 1	0.8	1.4	0.6	0.8	1.2	1.1	0.9	1.1
Notch 1	1.3	**2.5**	0.8	1.6	0.7	0.9	1.0	0.8
Notch 2	1.2	1.0	0.9	0.6	0.9	0.9	0.9	1.0
RAGE	1.5	**2.8**	1.0	1.8	0.7	0.9	1.0	0.9
TNF alpha	1.1	1.2	0.8	0.9	1.1	1.0	1.1	1.1
VEGF A	1.1	0.9	1.4	0.9	**2.1**	1.4	1.5	1.1
BDNF	1.3	**4.3**	0.8	**3.2**	1.2	1.7	1.1	0.9

Fold change in optical density for cytokines in Co-culture media samples vs. Media Only or FaDu only media. For groups where n = 2, median fold change is presented.

Supplementary Table 4. Characteristics of young adult male versus young adult female rat DRG neurons in Media Only

Media Only DRG Neurons - Young Adult Rats (3-5 mo)			
Characteristic	**Males**	**Females**	**Significance**
Current Threshold (pA)	266.5 ± 37.2 (31)	220.0 ± 33.5 (27)	n.s. (p=.36)
RMP (mV)	-57.1 ± 1.3 (31)	-55.0 ± 1.4 (27)	n.s. (p=.28)
AP Amplitude (mV)	115.9 ± 2.0 (31)	113.4 ± 2.9 (27)	n.s. (p=.47)
AP Rise Time (ms)	3.2 ± 0.3 (31)	3.6 ± 0.4 (27)	n.s. (p=.42)
AP Fall Time (ms)	15.2 ± 2.4 (31)	17.0 ± 1.8 (27)	n.s. (p=.56)
Width at 0 mV (ms)	8.0 ± 1.7 (30)	8.1 ± 0.8 (26)	n.s. (p=.96)
AP Overshoot (mV)	62.1 ± 2.5 (30)	62.9 ± 1.4 (26)	n.s. (p=.79)
AP Afterhyperpolarization (mV)	11.3 ± 1.5 (15)	10.3 ± 1.9 (13)	n.s. (p=.68)
Threshold Potential (mV)	-9.7 ± 2.2 (31)	-12.9 ± 1.6 (27)	n.s. (p=.26)
Response to Current Stimulation			$F_{1,55}$=3.3, n.s. (p=.07)
1X	0.5 ± 0.2 (26)	0.5 ± 0.3 (26)	
2X	1.6 ± 0.4 (26)	2.1 ± 0.3 (26)	
3X	2.9 ± 0.5 (26)	2.8 ± 0.5 (26)	

Data expressed as Mean ± SEM with the number of neurons in parentheses. RMP: resting membrane potential; AP: action potential