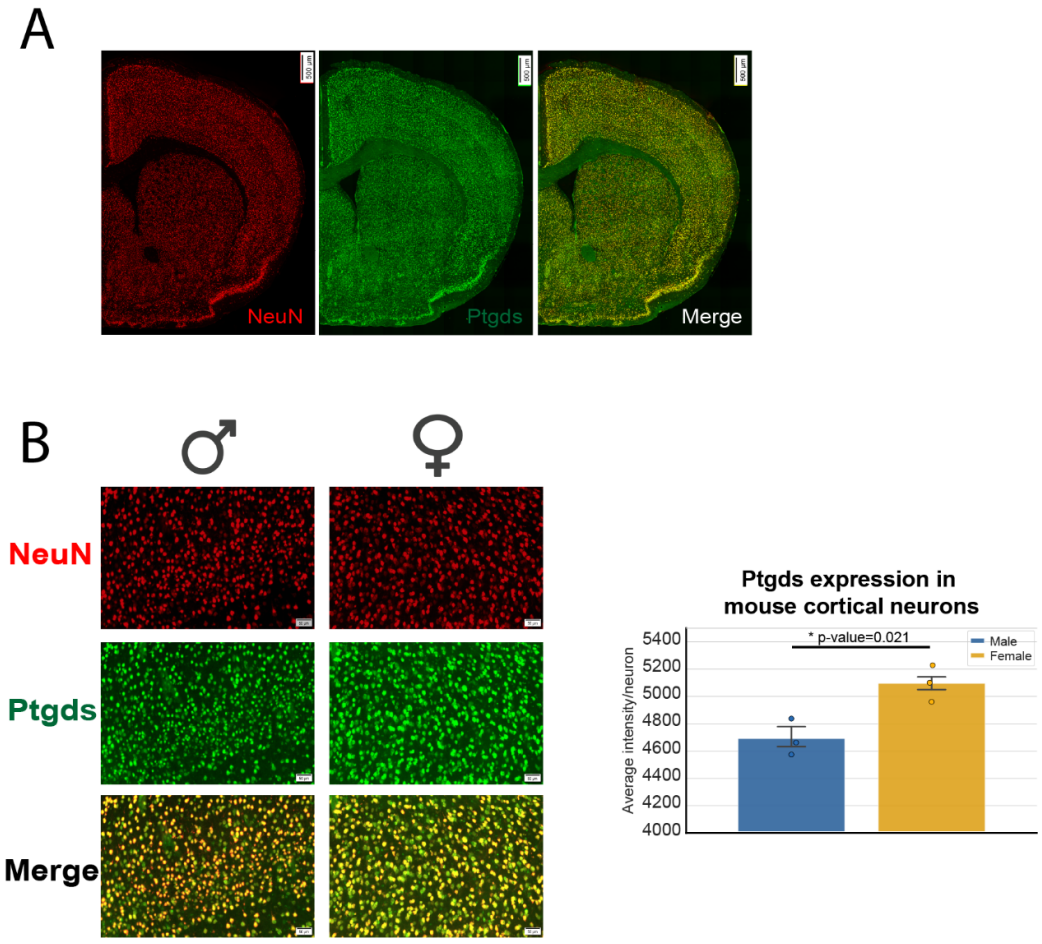
Sex Differences in Nociceptor Translatomes Contribute to Divergent Prostaglandin Signaling in Male and Female Mice

Supplement 1



Suppl. Figure S1: *Ptgds expression is higher in female cortical neurons.* **A**, Ptgds is highly co-localized with neuronal marker NeuN in the mouse brain. **B**, Ptgds expression in mouse cortical neurons is higher in females compared to males (* Unpaired t-test, t = 3.699, df = 4, p-value= 0.0209). A total of 100 neurons per slice (3 slices per animal) were used to quantify Ptgds. Panel A Scale bar = 500 µm; panel B scale bar = 50 µm.

Suppl. Table S1A: Raw transcripts per million for INPUT and respective percentiles.

Suppl. Table S1B: Quantile normalized transcripts per million for INPUT and respective statistics.

Suppl. Table S2A: Raw transcripts per million for IP and respective percentiles.

Suppl. Table S2B: Quantile normalized transcripts per million for IP and respective statistics.

Suppl. Table S3: Differentially expressed genes in INPUT.

Suppl. Table S4: Differentially translated mRNAs in IP.

See separate Excel file for Tables S1-S4.

Suppl. Table S5: Functional analysis of differentially expressed INPUT genes. (ND: No data available).

Gene name	Gene description	GO Biological Process	GO Cellular Component	GO Molecular Function	Nervous system; nociception related?	Reference
	UP-REGULATED IN FEMALES (INPUT)					
Hoxd4	Homeobox d4	Transcription regulation	Nuceloplasm; Cell junction	Transcription factor	Wallerian degeneration	PMID: 31397358
Smpd4	Sphingomyelin phosphodiesterase 4	Sphingomyelin catabolic process	Nucleus; Membrane; Golgi apparatus; Endoplasmic reticulum	Sphingomyelin phosphodiesterase activity	Loss of this protein is associated with microcephaly	PMID: 31495489
Vpreb3	Pre-b lymphocyte gene 3	Regulation of immunoglobulin secretion	Endoplasmic reticulum; extracellular space	*ND*	*ND*	
Bc051142	cDNA sequence bc051142	*ND*	Membrane	*ND*		
Runx1t1	Runx1 translocation partner 1	Transcription regulation	Nucleus	Transcription factor activity	Neuronal differentiation in hippocampus	PMID: 25473084
Adra2b	Adrenergic receptor, alpha 2b	MAPK casacade; Regulation of vasoconstriction; Regulation of muscle contraction and neuronal differentiation	Membrane	Adrenergic receptor activity; epinephrine binding	*ND*	
Srp19	Signal recognition particle 19	Srp-dependent cotranslational protein targeting to membrane	Nucleus; Signal recognition particle; Endoplasmic reticulum	7s RNA binding; ribosome binding	*ND*	
Lepr	Leptin receptor	Regulation of mapk cascade; signal transduction; protein phosphorylation	Membrane	Transmembrane signaling receptor activity	Neuropathic pain	PMC5651484; PMID: 28822786
Kng2	Kininogen 2	Regulation of endopeptidase activity; Regulation of cytosolic calcium concentration	Extracellular space	Endopeptidase inhibitor activity; Bradykinin precursor	*ND*	
Chrm5	Cholinergic receptor, muscarinic 5	Transmission of nerve impulse; Chemical synaptic transmission	Synapse; Membrane; Cell junction	G protein-coupled acetylcholine receptor activity; Extracellularly glycine-gated chloride channel activity	Opioid- or nausea/vomiting signaling pathways	PMID: 21570824
Anpep	Alanyl (membrane) aminopeptidase	Angiogenesis; Cell differentiation	Membrane; Cytoplasm; Extracellular space	Aminopeptidase activity	Complex regional pain syndrome	PMID: 24244504
Tmem230	Transmembrane protein 230	Synaptic vesicle transport	Membrane; Synaptic vesicle; autophagosome	*ND*	Parkinson's disease	PMID: 30175983
Scube3	Signal peptide, cub domain, egf-like 3	Regulation of smoothened signaling pathway	Membrane; Extracellular space	Calcium ion binding; protein binding		
Plcb4	Phospholipase c, beta 4	Modulation of chemical synaptic transmission; metabolic process	Cell; Nucleus; Cytoplasm; Postsynapse	Hydrolase activity; protein binding	Inflammatory pain	PMID: 12954872
Kif23	Kinesin family member 23	regulation of cytokinesis	Cell; Nucleoplasm	Microtubule binding; ATP binding	*ND*	
Bc049762	cDNA sequence Bc049762	*ND*	*ND*	*ND*	*ND*	
Ackr3	Atypical chemokine receptor 3	Cell adhesion; Immune response	Cell; Cytoplasm; Membrane; Nucleus	Scavenger receptor activity; Chemokine receptor activity	*ND*	

Gene name	Gene description	GO Biological Process	GO Cellular Component	GO Molecular Function	Nervous system; nociception related?	Reference
Sirpb1b	Signal-regulatory protein beta 1b	Cell-cell adhesion; phagocytosis; T cell activation	Membrane	*ND*	*ND*	
Pamr1	Peptidase domain containing associated with muscle regeneration 1	Proteolysis	Extracellular space	Endopeptidase activity; calcium ion binding	*ND*	
Jchain	Immunoglobulin joining chain	Immune response	Extracellular space	Immunogloblobulin binding	*ND*	
Pi16	Peptidase inhibitor 16	Peptidase activity	Extracellular space	Peptidase inhibitor activity	Fibroblast-derived protein involved in neuropathic pain	PMID: 32079726
Ncoa5	Nuclear receptor coactivator 5	Glucose homeostasis	Nucleoplasm; Nucleus	Chromatin binding	*ND*	
Snurf	SNRPN upstream reading frame	*ND*	Nucleus	ATPase binding	*ND*	
Trabd2b	Trab domain containing 2b	Wnt signaling pathway	Membrane	Hydrolase activity; Peptidase activity	*ND*	
Card6	Caspase recruitment domain family, member 6	I-KappaB kinase/nf-kappaB signaling; Apoptotic process	Cytoplasm	*ND*	Spinal cord injury; Reumathoid arthritis	PMID: 31841440; PMID: 32317187
Lbp	Lipopolysaccharide binding protein	Immune response	Membrane; Extracellular space	Lipopolysaccharide binding	*ND*	
Epb41l5	Erythrocyte membrane protein band 4.1 like 5	Epithelial cell morphogenesis	Membrane; Cytoplasm; Cell junction	Cytoskeletal protein binding	*ND*	
Map3k8	Mitogen-activated protein kinase kinase kinase 8	MAPK cascade; Immune system process	Cytoplasm	Kinase activity; Nucleotide binding	*ND*	
Dio2	Deiodinase, iodothyronine, type ii	Regulation of cold-induced thermogenesis; Hormone biosynthetic process	Membrane	Oxidoreductase activity	Osteoarthritis	PMID: 25693039
Nudt6	Nudix (nucleoside diphosphate linked moiety x)-type motif 6	Regulation of cell cycle	Cytoplasm	Hydrolase activity	*ND*	
Pkd2l2	Polycystic kidney disease 2-like 2 (also known as trpp5)	Ion transmembrane transport	Membrane	Calcium channel activity	*ND*	
Kdm5c	Lysine (k)-specific demethylase 5c	Transcription regulation; chromatin organization	Nucleus; cytoplasm	Transcription factor activity;histone demethylase activity	*ND*	
Pnoc	Prepronociceptin	Sensory preception	Neuronal cell body	Opioid peptide activity		
Eif2s3x	Eukaryotic translation initiation factor 2, subunit 3, structural gene x-linked	Formation of translation preinitiation complex	Eukaryotic translation initiation factor 2 complex in cytoplasm	Translation factor activity; RNA binding	*ND*	
Catip	Ciliogenesis associated ttc17 interacting protein	Cell projection organization	Cytoskeleton; membrane; cytoplasm; nucleus	*ND*	*ND*	
Arg1	Arginase	Immune response; metabolic process	Neuron projection; cytoplasm; extracellular space	Arginase activity	*ND*	
Cfap70	Cilia and flagella associated protein 70	Cillium movement and assembly	Axoneme	Protein binding	*ND*	
Tnfrsf11b	Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	Signal transduction; Extracellular matrix organization	Extracellular space	Protein binding	*ND*	

Gene name	Gene description	GO Biological Process	GO Cellular Component	GO Molecular Function	Nervous system; nociception related?	Reference
Dapl1	Death associated protein-like 1	Apoptotic signaling pathway	*ND*	Death domain binding	*ND*	
Cxcl16	Chemokine (c-x-c motif) ligand 16	T cell chemotaxis	Extracellular space; Membrane	Chemokine activity	Inflammatory pain	PMID: 22815815
Fam221a	Family with sequence similarity 221, member A	*ND*	*ND*	*ND*	*ND*	
	UP-REGULATED IN MALES (INPUT)					
Qrfpr	Pyroglutamylated rfamide peptide receptor	Neuropeptide signaling pathway; Metabolism and energy homeostasis	Membrane	Neuropeptide Y receptor activity	*ND*	
Spry1	Sprouty RTK signaling antagonist 1	MAPK activity; Regulation of fibroblast growth factor receptor signaling pathway	Cytoplasm; Nucleus; Membrane	Protein binding	*ND*	
BC030867 (Hrob)	Homologous recombination factor with ob-fold	Cellular response to DNA damage stimulus	Site of DNA damage	Single-stranded DNA binding	*ND*	
Pappa2	Pappalysin 2	Proteolysis	Apical plasma membrane; cytosol	Metallopeptidase activity	*ND*	
Lctl	Lactase-like	Response to stimuli; Metabolic process	*ND*	*ND*	*ND*	
Col10a1	Collagen, type x, alpha 1	Extracellular matrix organization	Extracellular space	Extracellular matrix structural constituent	Osteoarthritis	PMID: 20498197
Pclaf	Pcna clamp associated factor	Cellular response to DNA damage stimulus	Centrosome; Nucleus	Chromatin binding	*ND*	
Gp9	Glycoprotein 9 (platelet)	Cell adhesion; Homeostasis	Membrane	Protein binding	*ND*	
Foxd3	Forkhead box d3	Transcription regulation	Nucleus	Transcription factor activity	*ND*	
Mrap2	Melanocortin 2 receptor accessory protein 2	Energy homeostasis; Metabolic process	Membrane	Receptor regulator activity	*ND*	
Sfi1	Sfi1 homolog, spindle assembly associated	Regulation of phosphatase activity	Cytoplasm; Centrosome; Cytoskeleton	Phosphatase binding	*ND*	
Nmbr	Neuromedin B receptor	Neuropeptide signaling pathway	Membrane; Cytosol	Neuropeptide receptor activity	Itch transmission; Thermal stimuli perception	PMID: 29133874; PMID: 22723708
Jmjd8	Jumonji domain containing 8	Regulation of I-kappaB kinase/nf-kappaB signaling; Regulation of glycolytic process; Regulation of sprouting angiogenesis	Cytoplasm; Nucleus	*ND*	*ND*	
Dut	Deoxyuridine triphosphatase	Regulation of signaling receptor activity; Regulation of protein-containing complex assembly	Cytoplasm; Nucleus; Mithocondrion	Receptor inhibitor activity	*ND*	
Cd276	Cd276 antigen	Regulation of inflammatory response; Regulation of cytokine production; T cell receptor signaling pathway	Membrane	Signaling receptor binding	*ND*	
Srd5a2	Steroid 5 alpha-reductase 2	Sex differentiation; Testosterone biosynthetic process	Cytoplasm; Membrane	Testosterone biosynthetic process	*ND*	
Nat14	N-acetyltransferase 14	*ND*	Membrane	Transferase activity	*ND*	

Gene name	Gene description	GO Biological Process	GO Cellular Component	GO Molecular Function	Nervous system; nociception related?	Reference
Crtac1	Cartilage acidic protein 1	Axonal fasciculation; Regulation of receptor binding	Extracellular region	Calcium ion binding; Protein binding	Osteoarthritis	PMID: 27415616
Smtnl2	Smoothelin-like 2	Actin cytoskeleton organization	Microtubule organizing center	Protein binding		
Nrarp	Notch-regulated ankyrin repeat protein	Notch signaling pathway; Regulation of T cell differentiation; Regulation of canonical Wnt signaling pathway; Regulation of cell-cell adhesion	*ND*	Protein binding	*ND*	
Cdca8	Cell division cycle associated 8	Cell cycle and division	Cell	Protein binding		
Ep400	E1a binding protein p400	Chromatin organization	Nucleus	Nucleotide binding; Helicase activity	Role in myelination in CNS	PMID: 31081019
Fgf2	Fibroblast growth factor 2	Cell differentiation; regulation of fibroblast migration; angiogenesis	Extracellular space; cell; nucleus	Chemoattractant activity; growth factor activity	Bone cancer pain	PMID: 27506449
Fscn1	Fascin actin-bundling protein 1	Regulation of extracellular matrix; Regulation of extracellular matrix	Cytoplasm; cell-cell junction; Myelin sheath	Protein binding; actin binding	Inflammatory component in neuropathic pain	PMID: 29052088
Ky	Kyphoscoliosis peptidase	*ND*	*ND*	*ND*		
Chd7	Chromodomain helicase DNA binding protein 7	Central nervous system development; Chromatin organization	Nucleus	Protein binding; Helicase activity; Hydrolase activity	Neuron differentiation; CNS myelination	PMID: 28317875; PMID: 26928066
Atf3	Activating transcription factor 3	Transcription regulation	Nucleus	Transcription factor activity	Injury marker	PMID: 15066140
Grid2ip	Glutamate receptor, ionotropic, delta 2 (grid2) interacting protein 1	G protein-coupled glutamate receptor signaling pathway; Actin cytoskeleton organization; Long-term synaptic depression	Synapse; Membrane; Cell junction	Protein binding	*ND*	
Olfml3	Olfactomedin-like 3	Extracellular matrix glycoprotein	*ND*	*ND*	Glial cells	PMID: 30093905
Zfp420	Zinc finger protein 420	Immune system	*ND*	*ND*	Mutation in axonal Charcot-Marie-tooth-disease	PMID: 29341343
Hspb3	Heat shock protein 3	*ND*	Nucleus; Cytoplasm	*ND*		
Penk	Preproenkephalin	Chemical synaptic transmission; Sensory perception of pain; Glial cell proliferation; Response to morphine	Membrane; Axon; Synapse	Opioid peptide activity	Opioid system in a mice neuropathic pain model	PMID: 30176322
Rnasek	Ribonuclease, RNase K	Nucleic acid phosphodiester bond hydrolysis	Membrane	Nuclease activity	*ND*	
Ddx3y	Dead (asp-glu-ala-asp) box polypeptide 3, y-linked	Translation machinery	Nucleoplasm; P granule	Nucleic acid binding	*ND*	
Commd1	Comm domain containing 1	Protein transport; Regulation of plasma lipoprotein particle levels	Endosome; membrane; nucleus	Protein binding; Sodium channel inhibitor activity	*ND*	
Uts2b	Urotensin 2b	Regulation of blood vessel diameter; Signal transduction	Extracellular region	Hormone activity	*ND*	

Gene name	Gene description	GO Biological Process	GO Cellular Component	GO Molecular Function	Nervous system; nociception related?	Reference
Kdm5d	Lysine (k)-specific demethylase 5d	Regulation of androgen receptor signaling pathway	Nucleus	DNA binding	*ND*	
Uty	Ubiquitously transcribed tetratricopeptide repeat gene, y chromosome	Regulation of gene expression	Membrane	Protein binding	*ND*	
Eif2s3y	Eukaryotic translation initiation factor 2, subunit 3, structural gene y-linked	Translation machinery	Eukaryotic translation initiation factor 2 complex	Translation initiation factor activity	*ND*	

Suppl. Table S6: Functional analysis of differentially translated IP mRNAs. (ND: No data available)

Gene name	*Gene description*	*GO Biological Process*	*GO Cellular Component*	*GO Molecular Function*	*Nervous system, nociception related?*	*Reference*
	UP- REGULATED IN FEMALES					
Gm527	Predicted gene 527	*ND*	*ND*	*ND*	*ND*	
Pcdha8	Protocadherin alpha 8	Cell adhesion; nervous system development	Membrane	Calcium ion binding	May be involved in the establishment and maintenance of specific neuronal connections	PMID: 10380929
Ccdc17	Coiled-coil domain containing 17	*ND*	*ND*	*ND*	*ND*	
Lime1	Lck interacting transmembrane adaptor 1	Regulation of map kinase activity; T and B cell receptor signaling	Membrane	Protein kinase binding	*ND*	
Map3k1	Mitogen-activated protein kinase kinase kinase 1	Protein phosphorylation	*ND*	Protein kinase activity	Inflammatory pain	PMID: 18366630
Efcab7	Ef-hand calcium binding domain 7	Regulates hedgehog	*ND*	Calcium ion binding	*ND*	
Zmym1	Zinc finger mym-type containing 1	Transcription regulation	*ND*	Protein dimerization activity; zinc ion binding	*ND*	
Mllt10	Myeloid/lymphoid or mixed-lineage leukemia; translocated to, 10	Transcription regulation	Nucleus, cytosol	Protein binding	*ND*	
Gm42878	Predicted gene 42878	Protein phosphorylation		Protein kinase activity	*ND*	
Pf4	Platelet factor 4	Inflammatory/immune response; adenylate cyclase-activating g protein-coupled receptor signaling pathway	Cytoplasm	Cytokine/chemokine activity	Sickle cell disease	PMID: 2145991
Necab2	N-terminal ef-hand calcium binding protein 2	Positive regulation of ERK1 and ERK2 cascade	Cytoplasm, membrane, axon	Signaling scaffold protein	Inflammatory hypersensitivity; Nerve injury	PMID: 29893745; PMID: 24616509
Ep400	E1a binding protein p400	Chromatin organization	Nucleus	Nucleotide binding	Myelin formation	PMID: 31081019
Ccdc84	Coiled-coil domain containing 84	*ND*	*ND*	*ND*	Cluster headache	PMID: 28074859
Slc9a2	Solute carrier family 9 member A2	Sodium ion transport; protein localization; pH regulation	Membrane	Antiporter activity	*ND*	
Fbln1	Fibulin 1	Negative regulation of erk1 and erk2 cascade	Extracellular matrix	Protein binding	*ND*	
Klf4	Kruppel-like factor 4	Transcription regulation	Nucleus	Nucleic acid binding	Overexpression of KLF4 can regulate neuronal cell cycle proteins and sensitize neurons to NMDA.	PMID: 19041854
Inpp5d	Inositol polyphosphate-5-phosphatase d	Signal transduction	Cytoplasm	Protein binding	Alzheimer's disease	PMID: 30906402; PMID: 28482637
Dpt	Dermatopontin	Cell adhesion	Extracellular region	Extracellular matrix structural constituent	Potential role in neuronal functions *in vivo* (zebrafish)	PMID: 23266816
Hmgxb4	Hmg box domain containing 4	Transcription regulation	Nucleus	DNA binding	*ND*	
Eif2s3x	Eukaryotic translation initiation factor 2, subunit 3, structural gene x-linked	Formation of translation preinitiation complex	Eukaryotic translation initiation factor 2 complex in cytoplasm	Translation factor activity; RNA binding	*ND*	

Gene name	*Gene description*	*GO Biological Process*	*GO Cellular Component*	*GO Molecular Function*	*Nervous system, nociception related?*	*Reference*
Sfrp4	Secreted frizzled-related protein 4	Wnt signaling pathway	Cell	Protein binding	*ND*	
Rabepk	Rab9 effector protein with kelch motifs	*ND*	Membrane, endosome	Protein binding	ND	
Igfbp6	Insulin-like growth factor binding protein 6	Regulation of insulin-like growth factor receptor signaling pathway	Cytoplasm	Growth factor binding	Spinal cord injury, peripheral nerve injury	PMID: 27888466; PMID: 9762866
Zmynd8	Zinc finger, mynd-type containing 8	Modulation of excitatory postsynaptic potential	Nucleoplasm, dendrite	Protein binding	Neuronal differentiation	PMID: 20331974
Mtss1l	Mtss i-bar domain containing 2	Membrane organization	Membrane	Actin binding	Membrane curvature and dendritic spine formation, regulation of synapse currents	PMID: 31232686
Rab11b	Rab11b, member ras oncogene family	Rab protein signal transduction, protein transport	Synapse, cell, membrane	Non-kinase enzyme	Neuronal endosomal pathways	PMID: 14627637
Lepr	Leptin receptor	Regulation of MAPK cascade, signal transduction, protein phosphorylation	Membrane	Transmembrane signaling receptor activity	Neuropathic pain	PMID: 29071294; PMID: 28822786
Prelp	Proline arginine-rich end leucine-rich repeat	Cell aging	Extracellular region	Protein binding	Endometriosis	PMID: 28678915
Gjb6	Gap junction protein, beta 6	Gap junction assembly; Cell communication	Cell; Membrane; Gap junction	Gap junction channel activity		
Lbp	Lipopolysaccharide binding protein	Immune response	Membrane	Signaling receptor binding	Metabolic neuropathy	PMID: 31145897
Repin1	Replication initiator 1	*ND*	Cytosolic ribosome	Nucleic acid binding	*ND*	
Slc6a13	Solute carrier family 6 (neurotransmitter transporter, GABA), member 13	Neurotransmitter transport	Neuron projection; Membrane	Neurotransmitter binding, symporter activity	*ND*	
Ptgds	Prostaglandin d2 synthase	Nociception; Sleep regulation; Temperature regulation	Cytoplasm	Prostaglandin-D synthase activity	Nociception	PMID: 9892701

Gene name	*Gene description*	*GO Biological Process*	*GO Cellular Component*	*GO Molecular Function*	*Nervous system, nociception related?*	*Reference*
	UP- REGULATED IN MALES					
Mapk1ip1	Mitogen-activated protein kinase 1 interacting protein 1	*ND*	Cytoplasm	*ND*	*ND*	
Chka	Choline kinase alpha	Metabolic process	Cytoplasm	Kinase activity	Neuronal function	PMC2925531
Serpinb1b	Serine (or cysteine) peptidase inhibitor, clade b, member 1b	*ND*	Cytoplasm	Regulator of enzyme	*ND*	
Timp1	Tissue inhibitor of metalloproteinase 1	Signal transduction	*ND*	Regulator of enzyme	Neuropathic pain, nerve injury	PMID: 17033093; PMID: 21782897
Ccer2	coiled-coil glutamate-rich protein 2	*ND*	*ND*	*ND*	taxane-induced neuropathic pain	PMC5347688
Desi2	Desumoylating isopeptidase 2	Proteolysis	Cytoplasm	Enzyme activity	*ND*	
Itga11	Integrin alpha 11	Cell adhesion	Membrane	Collagen binding	*ND*	

Gene name	*Gene description*	*GO Biological Process*	*GO Cellular Component*	*GO Molecular Function*	*Nervous system, nociception related?*	*Reference*
Apln	Apelin	Signal transduction	Extracellular space	Signaling receptor binding	Neuropathic pain, Alzheimer's disease	PMC5562064; PMID: 29807653
Bcat1	Branched chain aminotransferase 1, cytosolic	Amino acid byosynthetic and metabolic process	Cytoplasm	Catalytic activity	mTOR pathway in Alzheimer's disease	PMID: 29802157
Chac1	Chac, cation transport regulator 1	Notch signaling pathway, neurogenesis, regulation of protein processing	Cytoplasm	Gamma-glutamylcyclotransferase activity; notch binding	*ND*	
Hoxd11	Homeobox d11	Transcription regulation	Nucleus	DNA binding	*ND*	
Crym	Crystallin, mu	Transcription regulation	Nucleus, cytoplasm	Hormone binding, NADP binding	*ND*	
Fgf9	Fibroblast growth factor 9	Wnt signaling; MAPK cascade; cell-cell signaling	Cytoplasm	Fibroblast growth factor receptor binding	Neuron-derived fgf9	PMID: 19232523; PMID: 31726090
Sema6a	Sema domain, transmembrane domain (tm), and cytoplasmic domain, (semaphorin) 6a	Neuron migration, axon guidance, regulation of ERK1 and ERK2 cascade	Axon, membrane	*ND*	Axon guidance and neuronal connectivity; burn injury	PMID: 27392094; PMID: 27573516
Tbl3	Transducin (beta)-like 3	rRNA processing, post-transcriptional regulation	Pwp2p-containing subcomplex of 90s preribosome	Protein binding, snoRNA binding	*ND*	
Esam	Endothelial cell-specific adhesion molecule	Cellular protein localization, cell-adhesion	Membrane	*ND*	*ND*	
Pde8b	Phosphodiesterase 8b	Signal transduction	Hydrolase activity, metal ion binding	Enzyme	Phosphodiesterases in neurodegenerative diseases	PMID: 23129425
Ddx4	Dead (asp-glu-ala-asp) box polypeptide 4	Regulation of protein localization, male meiosis	Nucleus, cytoplasm, p granule, ribonucleoprotein complex	RNA binding	*ND*	
Nhlh2	Nescient helix loop helix 2	Transcription regulation	Nucleus	Protein binding	Neuronal transcription factor	PMID: 31421827
Ccne2	Cyclin e2	Synapsis	Nucleus, cytoplasm	Protein kinase activity		
Thrb	Thyroid hormone receptor beta	Transcription regulation	Nucleus	Protein binding	Neuronal regeneration after brain injury	PMID: 29378220
Hoxd10	Homeobox d10	Peripheral nervous system neuron development, transcription regulation	Cytoplasmic ribonucleoprotein granule		*ND*	
Slc16a13	Solute carrier family 16 (monocarboxylic acid transporters), member 13	Transmembrane transport	Membrane	Symporter activity, GABA transporter	*ND*	
Serpina3c	Serine (or cysteine) peptidase inhibitor, clade a, member 3c	Regulation of peptidase activity, response to cytokine	Extracellular space	Peptidase inhibitor activity	Neuropathic pain	PMID: 25915831
Card9	Caspase recruitment domain family, member 9	Positive regulation of interleukin-6 production, regulation of stress-activated MAPK cascade, JNK cascade	Cytoplasm	Protein binding	*ND*	
Atf3	Activating transcription factor 3	Transcription regulation	Nucleus	*ND*	Injury marker	PMID: 15066140
Gtpbp4	GTP binding protein 4	Ribosome biogenesis, protein stabilization, cell-cell adhesion	Nucleus, cytoplasm, membrane	GTP activity	*ND*	
Sez6l	Seizure related 6 homolog like	Regulation of protein kinase C signaling, synapse maturation	Membrane, neuronal cell body	Protease substrate in neurons	Burn injury; neuropathic pain	PMID: 27573516; PMID: 25880204

Gene name	*Gene description*	*GO Biological Process*	*GO Cellular Component*	*GO Molecular Function*	*Nervous system, nociception related?*	*Reference*
Srd5a2	Steroid 5 alpha-reductase 2	Regulation of testosterone; androgen processing		Enzyme	*ND*	
Kdm5d	Lysine (k)-specific demethylase 5d	Regulation of androgen receptor signaling pathway	Nucleus	DNA binding	*ND*	
Uty	Ubiquitously transcribed tetratricopeptide repeat gene, y chromosome	Regulation of gene expression	Membrane	Protein binding	*ND*	
Ddx3y	Dead (asp-glu-ala-asp) box polypeptide 3, y-linked	Translation machinery	Nucleoplasm, P granule	Nucleic acid binding	*ND*	
Eif2s3y	Eukaryotic translation initiation factor 2, subunit 3, structural gene y-linked	Translation machinery	Eukaryotic translation initiation factor 2 complex	Translation initiation factor activity	*ND*	

Suppl. Methods and Materials

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of University of Texas at Dallas protocol number 14-04.

Nav1.8Cre/Rosa26^{fstrap} mice**:** Rosa26^{fsTRAP} mice were purchased from The Jackson Laboratory (stock #022367). Transgenic mice expressing Cre recombinase under the control of the Scn10a (Nav1.8) promoter were obtained initially from Professor John Wood (University College London) but are commercially available from Infrafrontier (EMMA ID: 04582). Initial studies demonstrated that the introduction of the Cre recombinase in heterozygous animals does not affect pain behavior, and their DRG neurons have normal electrophysiological properties (1). Nav1.8-cre mice on a C57BL/6J genetic background were maintained and bred at the University of Texas at Dallas. Upon arrival, Rosa26^{fsTRAP} mice were crossed to Nav1.8-cre to generate the Nav1.8-TRAP mice that express a fused EGFP-L10a protein in Nav1.8-positive neurons. TRAP experiments were performed using male only and female only Nav1.8-TRAP littermates 8–12 weeks old. Mice were group housed (4 maximum) in non-environmentally enriched cages with food and water ad libitum on a 12 h light-dark cycle. Room temperature was maintained at 21 ± 2°C. Immunohistochemistry and ELISA assays were performed on C57BL/6J mice. Behavioral experiments were performed on C57BL/6J mice and ICR mice (we did not observe any strain differences).

TRAP

Nav1.8-TRAP male and female mice (3 mice per sex were pooled for each of 4 biological replicates) were decapitated under isoflurane anesthesia and DRGs rapidly dissected in ice-cold dissection buffer (1× HBSS (Invitrogen, 14065006), 2.5 mM HEPES-NaOH [pH 7.4], 35 mM Glucose, 5mM MgCl2, 100 µg/ml cycloheximide, 0.2 mg/ml emetine). DRGs were transferred to ice-cold Precellys Tissue homogenizing CKMix tube with lysis buffer (20 mm

HEPES, 12 mm MgCl2, 150 mm KCl, 0.5 mm DTT, 100 µg/ml cycloheximide, 20 µg/ml emetine, 80 U/ml SUPERase IN, Promega, 1 µl DNase, and protease inhibitor). The lysate was prepared by homogenizing the samples using Precellys® Minilys Tissue Homogenizer at 10 second intervals for a total of 80 seconds, in the cold room (4°C). Samples were then centrifuged at 2000 × g for 5 min to prepare postnuclear fractions. Next, 1% NP-40 and 30 mM 1,2-dihexanoyl-sn-glycero-3-phosphocholine were added and samples centrifuged at 15,000 × g for 10 min to generate a postmitochondrial fraction. A 200 µl sample of this fraction was saved for use as INPUT (bulk RNA-sequencing), and the remaining was incubated with protein G-coated Dynabeads (Invitrogen) bound to 50 µg anti-GFP antibodies (HtzGFP-19F7 and HtzGFP-19C8, Memorial Sloan Kettering Centre) for 3 h at 4°C with end-over-end mixing. Anti-GFP beads were washed with high salt buffer (20 mm HEPES, 5 mm MgCl2, 350 mm KCl, 1% NP-40, 0.5 mm DTT, and 100 µg/ml cycloheximide), and RNA was eluted from all samples using the Direct-zol kit (Zymo Research) according to the manufacturer's instructions. RNA yield was quantified using a Nanodrop system (Thermo Fisher Scientific), and RNA quality was determined by fragment Analyzer (Advanced Analytical Technologies).

Immunohistochemistry

Animals were anesthetized with isoflurane (4%) and euthanized by decapitation and tissues were flash frozen in OCT on dry ice. Sections of DRG (20 µm) and brain (20 µm) were mounted onto SuperFrost Plus slides (Thermo Fisher Scientific) and immediately fixed in ice-cold formalin (10%) for 15 min followed by dehydration in 50% ethanol, 70% ethanol and 100% ethanol at room temperature for 5 min each. Tissues were briefly air dried and boundaries were drawn around each section using a hydrophobic pen (ImmEdge H-4000). Once dry, the sections were blocked for at least 1 h in 10% normal goat serum with 0.3% TX-100. DRG slices were stained with peripherin, NeuN and Ptgds overnight at 4°C and brain slices were stained with NeuN and Ptgds for 2 h at room temperature. The peripherin antibody (P5117) was obtained from Sigma-Aldrich, NeuN (MAB377) was obtained from Millipore Sigma and Ptgds (ab182141) from Abcam. Sections were then washed and incubated with respective Alexa Fluor secondary antibodies for 1 h at room temperature. Sections were washed, air dried, and then coverslipped with Prolong Gold Antifade reagent (Fisher Scientific; P36930).

DRG images were taken using an Olympus FluoView 1200 confocal microscope, using the same settings for all images. Analysis of DRG images was done using ImageJ version 1.48 (National Institutes of Health, Bethesda, MD). The values plotted on Figure 5E were obtained by calculating the corrected total cell fluorescence (CTCF) using the following formula: CTCF= Integrated Density – (Area of selected cell X Mean fluorescence of background readings).

Brain images were taken using Olympus vs120 virtual slide microscope, using the same settings for all images. Analysis of brain images was performed using Olympus cellSens software. An ROI of the same size was placed over 100 randomly selected cortical neurons per section to measure the mean gray intensity value. 3 sections were analyzed per animal. Background fluorescence was measured similarly using a negative control that was only exposed to blocking solution and secondary antibodies (no primary). After subtracting background values of negative control, we averaged all intensity values (first per slice and second per animal) to obtain the values plotted on Suppl. Figure 1.

Ptgds validation and estrous cycle assessment

A separate TRAP experiment was conducted where we validated the expression of Ptgds bound to ribosome. We followed the same protocol as described above (TRAP) with two exceptions: we used only one mouse per replicate, and we tracked the female estrous cycle.

The stage of the cycle was determined by vaginal lavage followed by cytological evaluation according to a previous published protocol (2).

ELISA assay

PGD_2 levels in the DRG were evaluated using Prostaglandin D2-MOX Express ELISA Kit (Cayman, 500151) following the manufacturer's instructions. The same number of DRGs (collected from all levels cervical, thoracic and lumbar) were used per sample in the ELISA assay.

Injections

PGE_2 (Cayman, 14010) was diluted in sterile PBS and injected with a volume of 25 µl via a 30.5-gauge needle and given intraplantarly.

AT-56 (Tocris, 3531) was diluted in 10% DMSO and 30% cyclodextrine and administered intraperitoneally.

Behavior testing

All behavioral experiments were performed between 8:00 A.M. and 6:00 P.M. Facial grimacing was evaluated using the Mouse Grimace Scale (MGS) as described previously (3). Mechanical paw withdrawal thresholds were measured using the up-down method (4) with calibrated Von Frey filaments (Stoelting).

Library generation and sequencing

After purifying the RNA, cDNA libraries were prepared with total RNA Gold library preparation (with ribosomal RNA depletion) for all samples according to the manufacturer's instructions (Illumina). Quality control was performed for RNA extraction and cDNA library preparation steps with Qubit (Invitrogen) and High Sensitivity NGS fragment analysis kit on the Fragment Analyzer (Agilent Technologies). After standardizing the amount of cDNA per sample, the libraries were sequenced on Illumina NextSeq500 sequencing machine with 75-bp single-end reads. mRNA library preparation and sequencing was done at the Genome Center in the University of Texas at Dallas Research Core Facilities.

Mapping and TPM quantification:

RNA-seq read files (fastq files) were checked for quality by FastQC (Babraham Bioinformatics, https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and read trimming was done based on the Phred score and per-base sequence content. Trimmed Reads were then mapped against the reference genome and transcriptome (Gencode vM16 and GRCm38.p5) using STAR v2.2.1 (5). Relative abundances in Transcripts Per Million (TPM) for every gene of every sample was quantified by stringtie v1.3.5 (6). Non-coding genes and mitochondrial genes were removed from the analysis (based on Gencode annotation) and the TPMs for non-mitochondrial coding genes were re-normalized to sum to 1 million.

Order statistics and re-normalization of expression data

In order to identify a set of consistently expressed genes in the transcriptome (INPUT) samples, percentile ranks were calculated on TPMs for each coding gene for each sample. We conservatively chose 15,072 genes that were above the 30th percentile in each INPUT sample, for at least one sex, to be in the set of genes considered consistently detected in the transcriptome. Quantile normalization was then performed based on the set of all coding genes.

The IP (translatome) analysis was only performed for the 15,072 consistently transcriptome-expressed genes. In order to identify a set of consistently expressed genes in the translatome (IP) samples, percentile ranks were calculated on TPMs for each of the 15,072 consistently transcriptome-expressed coding genes for each sample. We then chose 12,542 genes out of those 15,072 genes to be consistently detected in the translatome based on whether their expression was on or above the 15th percentile (out of 15,072 genes) in each IP sample, for at least one sex.

The percentile thresholds for choosing consistently transcriptome-expressed and translatome-expressed genes were conservatively estimated by identifying thresholds that would eliminate genes with consistently low or no detected reads.

Differential expression analysis

We first calculated the log2-fold change (based on median TPMs) for each consistently transcriptome-expressed coding gene in the INPUT, and for each consistently translatome-expressed coding gene in the IP. It is calculated as follows:

$$\mathrm{LFC}_i^{(T)} = log_2\left(\frac{\mu_{i,f}^{(T)} + 0.01}{\mu_{i,m}^{(T)} + 0.01}\right)$$

where for gene i, LFC(T)i is the log2-fold change, μ(T)i,f and μ(T)i,m are the median TPMs in females and males respectively, with 0.01 as the smoothing factor.

We used strictly standardized mean difference (SSMD) (6, 7) to discover genes with systematically altered expression percentile ranks between males and females. SSMD is the difference of means controlled by the variance of the sample measurements. We used SSMD as a measure of effect size since it is appropriate for smaller sample sizes while simultaneously controlling for within-group variability. It is calculated as follows:

$$\mathrm{SSMD}_i^{(P)} = \frac{\mu_{i,f}^{(P)} - \mu_{i,m}^{(P)}}{\sqrt{v_{i,f}^{(P)} + v_{i,m}^{(P)}} + 0.01}$$

where for gene i, SSMD(P)i is the strictly standardized mean difference, with μ(P)i,f , v(P)i,f and μ(P)i,m , v(P)i,f are the means and variances of gene TPM percentile ranks in females and males respectively, under the assumption that covariance is 0, and with 0.01 as the smoothing factor.

For calculating the overlap in distribution between the qTPMs, in animals sampled from different sexes, we further calculated Bhattacharyya distance (8), which is used to calculate the amount of overlap in the area under the curve of the two sample distributions (corresponding to each sex) in order to identify the best candidates for the DE gene set. Unlike SSMD, BD does not make assumptions of equal variance in the two compared samples, and thus, is useful for comparing distributions of gene relative abundance (in TPMs). It is calculated as follows:

$$\mathrm{BD}_i^{(Q)} = \frac{1}{4}\left(\frac{(\mu_{i,f}^{(Q)} - \mu_{i,m}^{(Q)})^2}{v_{i,f}^{(Q)} + v_{i,m}^{(Q)} + 0.01}\right) + \frac{1}{4}\ln\left(\frac{1}{4}\left(\frac{v_{i,f}^{(Q)}}{v_{i,m}^{(Q)} + 0.01} + \frac{v_{i,m}^{(Q)}}{v_{i,f}^{(Q)} + 0.01} + 2\right)\right)$$

where for gene i, BD(Q)i is the Bhattacharyya Distance over gene qTPMs, with μ(Q)i,f , v(Q)i,f and μ(Q)i,m , v(Q)i,f are the means and variances of gene qTPMs in females and males respectively, and with 0.01 as the smoothing factor. The Bhattacharyya coefficient BC(Q)i ranges between 0 (for totally non-overlapping distributions) and 1 (for completely identical distributions) and is derived from the Bhattacharyya distance as follows:

$$\mathrm{BC}_i^{(Q)} = e^{-\mathrm{BD}_i^{(Q)}}$$

In our analysis, we used a modified form of the Bhattacharyya coefficient that ranges between 0 (for completely identical distributions) and +1 or -1 (for totally non-overlapping distributions, sign defined by the log-fold change value). It is calculated as follows:

$$(1 - \mathrm{BC}_i^{(Q)}) \times \mathrm{sgn}(\mathrm{LFC}_i^{(T)})$$

Motif analysis

For each gene in the up-regulated translation gene list (IP fraction), we created one 5′ UTR and 3’ UTR sequence per gene. For each gene, this was done by first extracting the respective UTR sequences from all isoforms from BioMart (7). Isoforms with UTRs <20 bases, or with sequence that is part of an ORF in another isoform were discarded. The remaining isoforms were collapsed into a single sequence in order of their location in the genome, with overlapping regions present exactly once in the sequence. The motif finding tool MEME (8) was used to identify motifs.

Network of interactions

We used STRING database (9) and Cytoscape (10) to generate Figure 3G and visualize interactions between genes differentially expressed and DRG enriched genes.

Single cell data

Single cell mouse DRG sequencing data from previously published work (11) was used to generate Figure 5B. Seurat package 2.2.1 (12) was used to cluster the single-cell data and visualization (t-SNE) (13).

Statistics

Statistical analysis for behavior, image quantification and ELISA assay was done in GraphPad Prism 8. Data visualization was done in Python (version 3.7 with Anaconda distribution). All data are represented as mean ± SEM. Single comparisons were performed using Student’s *t* test, and multiple comparisons were performed using a one-way or two-way ANOVA with *post hoc* tests for between-group comparisons. Statistical results can be found in the figure legends.

Coding for bioinformatics analysis and data visualization was done in Python (version 3.7 with Anaconda distribution).

Figure 1 and other schematic drawings were generated using Biorender (BioRender.com).

Data availability

Sequencing raw data files from this study are available in the NCBI GEO database under the accession number GSE155676.

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