Supplemental Digital Content 1: Supplemental Tables and Figures

Table ST1: Top 50 genes enriched in pDyn neuron nuclei compared to unidentified non-pDyn spinal nuclei

Gene	Log2FC[1]	Wald test statistic	Q-value[2]	Maximum normalized expression
Gucy2d	6.81	17.30	4.85E-64	862.26
Pdyn	6.00	6.34	6.01E-09	501.03
Gal	5.42	11.34	1.34E-27	256.60
Nhlh1	4.85	4.53	7.86E-05	38.44
Robo3	4.50	13.79	9.54E-41	566.18
Hrnr	4.39	4.99	9.35E-06	35.32
Pkd1l2	4.30	12.76	6.21E-35	736.27
Chodl	4.20	9.70	2.74E-20	137.13
Pnoc	4.16	5.93	6.94E-08	335.55
Olfr920	4.12	9.48	2.09E-19	184.50
Sstr2	4.00	14.50	4.96E-45	447.75
Kctd19	3.95	4.32	1.90E-04	30.46
Rasgrp1	3.93	15.59	4.49E-52	527.74
Prdm8	3.75	9.20	2.42E-18	152.07
Col6a6	3.72	9.35	6.45E-19	318.53
Col6a1	3.67	19.22	7.24E-79	3224.64
Adra2b	3.60	7.01	7.52E-11	79.25
Sstr1	3.48	9.80	9.96E-21	220.19
Hrh3	3.48	7.95	8.73E-14	220.19
Npbwr1	3.46	6.90	1.62E-10	87.26
Nos1	3.39	12.60	5.03E-34	1132.26
Cartpt	3.38	5.52	6.67E-07	82.05
Insrr	3.34	4.78	2.54E-05	42.64
Il20ra	3.30	8.27	6.85E-15	119.47
Neurod2	3.29	7.10	4.15E-11	102.81
Hpse2	3.28	5.87	9.73E-08	67.53
Hmga2	3.25	15.79	2.39E-53	603.12
Myo3b	3.24	10.62	3.08E-24	248.91
Slc26a4	3.24	6.29	7.97E-09	68.57
Hcrtr2	3.17	4.46	1.01E-04	298.15
Htr5b	3.15	4.65	4.63E-05	39.78
Skor1	3.15	9.81	9.04E-21	390.76
Abi3bp	3.08	15.26	6.54E-50	700.20
Vwa5b1	3.06	9.78	1.18E-20	481.28
Col24a1	3.05	5.53	6.28E-07	1934.68
Pou4f1	3.00	8.93	2.68E-17	196.43
Pax8	2.99	10.43	2.17E-23	1068.80

Asic4	2.97	7.88	1.40E-13	144.47
4930555F03Rik	2.95	10.31	6.81E-23	739.67
Rspo3	2.92	9.14	4.30E-18	169.21
Pcdh8	2.92	9.80	1.03E-20	369.84
Tacr3	2.86	12.09	2.44E-31	440.48
C130060K24Rik	2.85	4.78	2.56E-05	39.48
Trpc4	2.80	9.11	5.81E-18	308.09
Sfrp1	2.80	10.63	2.59E-24	349.13
Eepd1	2.79	11.21	5.33E-27	511.91
Slc35d3	2.78	8.58	5.70E-16	212.35
Abcc2	2.74	4.08	4.80E-04	43.51
Bhlhe22	2.72	7.07	5.13E-11	85.78
Zmat4	2.72	8.90	3.44E-17	861.22

[1] Log2FC = Mean $\log_2$(Fold change)

[2] Q-value = p-value adjusted for false discovery rate (FDR)

n = 4

Table ST2: Pain-related genes enriched ≥ 2-fold in pDyn nuclei compared to unidentified non-pDyn spinal nuclei

Gene	Log2FC[1]	Wald test Statistic	Q-value[2]	Max. Normalized Expression	Functional Class
Pdyn	6.00	6.34	6.01E-09	501.03	Neuropeptide
Gal	5.42	11.34	1.34E-27	256.60	Neuropeptide
Pnoc	4.16	5.93	6.94E-08	335.55	Neuropeptide
Hrh3	3.48	7.95	8.73E-14	220.19	Neuropeptide
Nos1	3.39	12.60	5.03E-34	1132.26	Enzyme
Bhlhe22	2.72	7.07	5.13E-11	85.78	Transcription Factor
Ret	2.41	10.19	2.43E-22	743.23	Enzyme-linked receptor
Adra2c	2.38	5.53	6.19E-07	103.89	GPCR
Th	2.35	6.52	1.89E-09	93.12	Enzyme
Htr1a	2.23	7.31	1.00E-11	188.49	GPCR
Kcnk2	2.17	8.54	7.96E-16	397.84	Voltage-gated channel
Nox4	2.02	7.95	8.84E-14	237.90	Enzyme
Glra2	2.01	5.88	9.32E-08	197.68	Ligand-gated channel
Penk	1.99	9.27	1.35E-18	1128.21	Neuropeptide
Sstr4	1.97	3.65	2.28E-03	43.91	GPCR
Grin2d	1.97	7.46	3.40E-12	1154.18	Ligand-gated channel
Grm2	1.93	4.33	1.83E-04	69.61	GPCR
Ntsr1	1.92	4.25	2.45E-04	57.83	GPCR
Gad2	1.89	7.74	4.11E-13	999.10	Enzyme
Slc32a1	1.82	6.03	3.76E-08	282.57	Transporter
Grik1	1.78	9.65	4.23E-20	1677.14	Ligand-gated channel
Agtr2	1.68	4.44	1.12E-04	113.24	GPCR
Oprk1	1.68	6.54	1.75E-09	548.32	GPCR
Kcnj3	1.52	7.82	2.36E-13	1091.57	Voltage-gated channel
Gria1	1.46	8.24	8.97E-15	2971.86	Ligand-gated channel
Gpr3	1.43	2.85	2.51E-02	52.22	GPCR
Stx1a	1.41	5.57	5.02E-07	199.46	Other
Tacr1	1.34	3.72	1.82E-03	169.21	GPCR
Kcnj6	1.33	6.10	2.59E-08	773.22	Voltage-gated channel
Cacna1g	1.32	5.81	1.35E-07	3551.89	Voltage-gated channel
Oprd1	1.31	3.45	4.30E-03	110.65	GPCR
Egr1	1.29	5.43	1.04E-06	330.70	Transcription Factor
Grm5	1.27	6.32	6.68E-09	4411.03	GPCR
Rgs4	1.21	4.70	3.61E-05	208.87	Other
Hoxb8	1.19	5.33	1.75E-06	623.13	Transcription Factor
Uchl1	1.18	4.97	1.04E-05	398.60	Enzyme
Chrna4	1.13	4.21	2.94E-04	406.96	Ligand-gated channel

Drd2	1.11	4.21	2.94E-04	150.64	GPCR
L1cam	1.09	4.98	9.91E-06	1537.87	Other
Adcy8	1.09	5.20	3.35E-06	1007.81	Enzyme
Cacna1h	1.08	7.09	4.41E-11	1497.79	Voltage-gated channel
Cacna1e	1.08	5.26	2.46E-06	8164.42	Voltage-gated channel
Cacnb3	1.07	4.25	2.50E-04	314.18	Other
Nptx1	1.06	4.84	1.93E-05	999.57	Other
Gabrg2	1.03	3.75	1.58E-03	585.92	Ligand-gated channel
Ece2	1.02	3.86	1.11E-03	1452.53	Enzyme
Oprm1	1.01	4.16	3.52E-04	328.28	GPCR

[1] Log2FC = Mean $\log_2$(Fold change)

[2] Q-value = p-value adjusted for false discovery rate (FDR)

n = 4

Table ST3: Genes enriched ≥ 2-fold in pDyn nuclei whose expression increased or decreased by ≥ 2-fold between any two time points[1].

GeneName	Log2FC[2]	Likelihood Ratio Statistic	Q-value[3]	Mean Normalized Expression			Cluster
				P7	P21	P80	
Gucy2d	6.41	300.19	1.55E-62	205.93	706.34	773.00	A
Prok2	4.09	13.88	9.30E-03	27.38	8.85	10.50	B
Npbwr1	3.99	42.62	2.48E-08	140.63	58.00	79.31	B
Nhlh1	3.58	12.23	1.83E-02	40.26	14.00	23.32	B
Htr5b	2.77	10.67	3.44E-02	12.87	24.04	31.64	A
Abcc2	2.12	46.59	4.12E-09	98.43	25.55	35.73	B
Neurod6	2.11	32.59	2.49E-06	95.14	41.77	45.40	B
Slc4a9	1.88	24.38	1.01E-04	42.33	15.21	14.26	B
Th	1.77	32.41	2.70E-06	36.24	99.30	85.95	A
Celsr1	1.76	16.34	3.33E-03	366.05	167.83	181.37	B
Hsf4	1.54	69.58	7.77E-14	16.85	56.62	82.48	A
Chgb	1.53	118.98	3.98E-24	854.56	1533.03	1877.51	A
Vgf	1.29	70.63	4.73E-14	115.04	145.01	306.68	A
Gpr141	1.28	32.65	2.43E-06	5.40	18.74	34.78	A
Npy	1.26	11.99	2.02E-02	64.54	29.01	39.88	B
Hcrtr1	1.23	60.64	5.69E-12	111.29	63.74	31.18	D
Cpa6	1.09	11.66	2.30E-02	11.14	25.98	24.91	A
Pcsk1	1.08	146.6	6.50E-30	176.25	288.91	514.67	A

[1] This table lists only genes meeting the stated criteria that were assigned to one of the select functional classes listed in the manuscript.

[2] Log2FC = Mean $\log_2$(Fold change) across all ages.

[3] Q-value = p-value of the likelihood ratio test adjusted for false discovery rate (FDR).

P7: n = 4, P21: n = 3, P80: n = 4

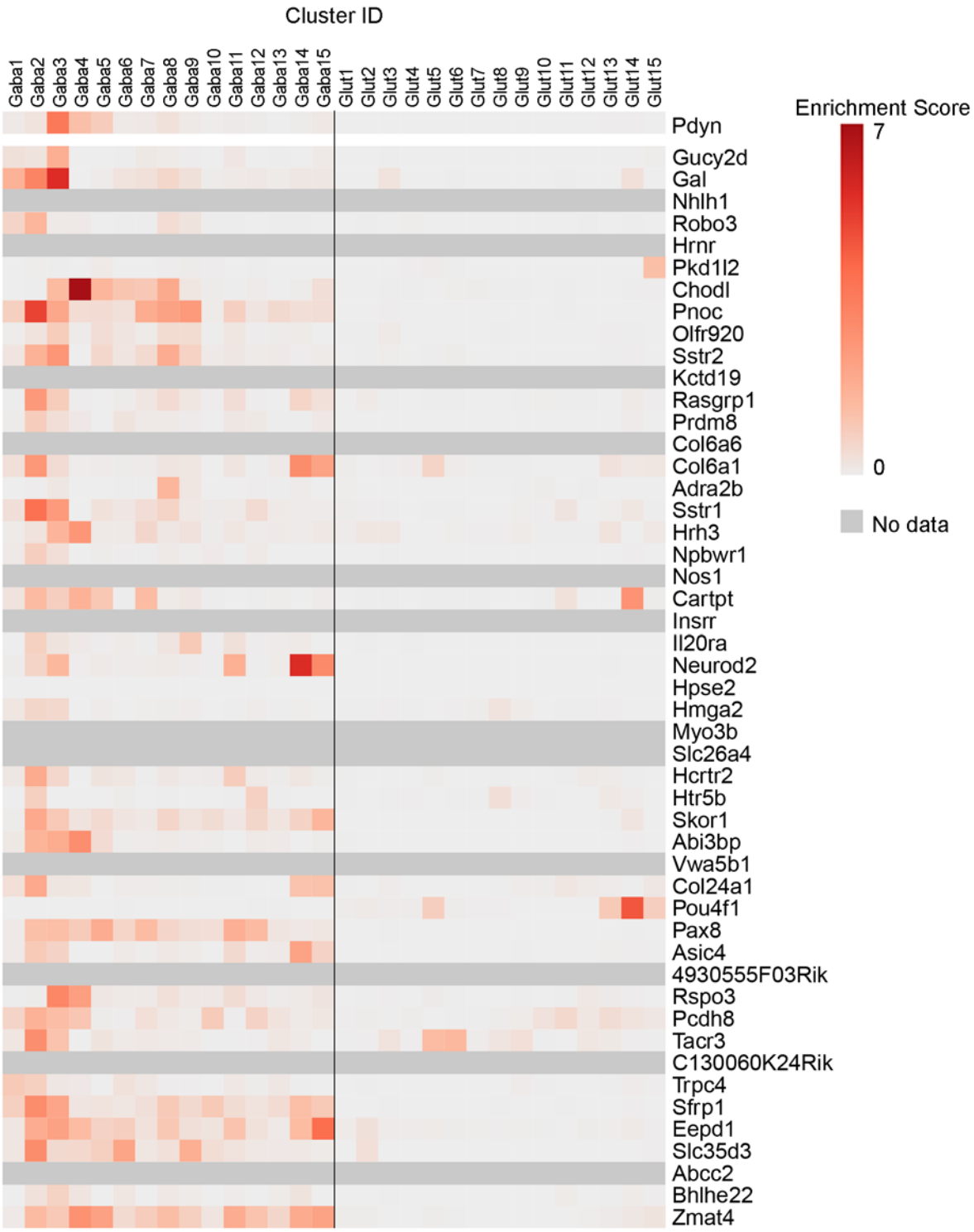

Figure S1: Enrichment scores per cluster (Häring et al.) of Top 50 genes enriched in pDyn neurons. The top 50 genes enriched in adult pDyn nuclei were compared to single-cell data obtained by Häring et al. [41]. Heat map shows enrichment score calculated by Häring et al. for each gene in both inhibitory (Gaba1-15) and excitatory (Glut1-15) neuron clusters identified in that study. Dark gray shading indicates that no enrichment score was provided for these genes.

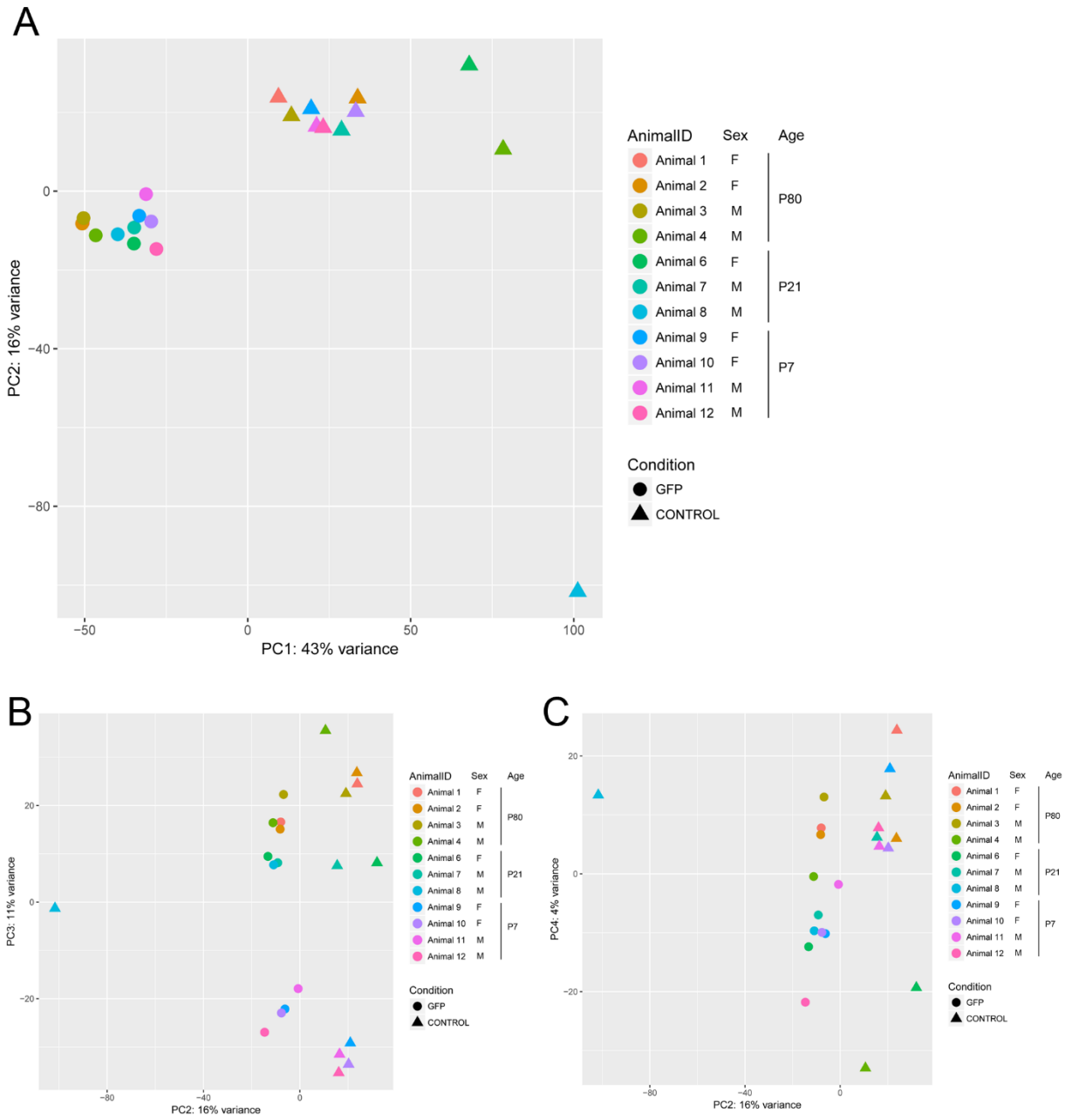

Figure S2: Principal Component Analysis (PCA) of Sorted Nuclei Samples. (A) PC1 vs PC2 plot shows two distinct clusters which correspond to GFP+ (circles) and GFP- samples (triangles). (B) PC2 vs PC3 plot shows less distinct clusters, but the samples primarily segregate based on the age of the animal. (C) PC2 vs PC4 plot shows minimal clustering. None of these PCA comparisons indicate the presence of sub-clusters corresponding to the sex of the animal.

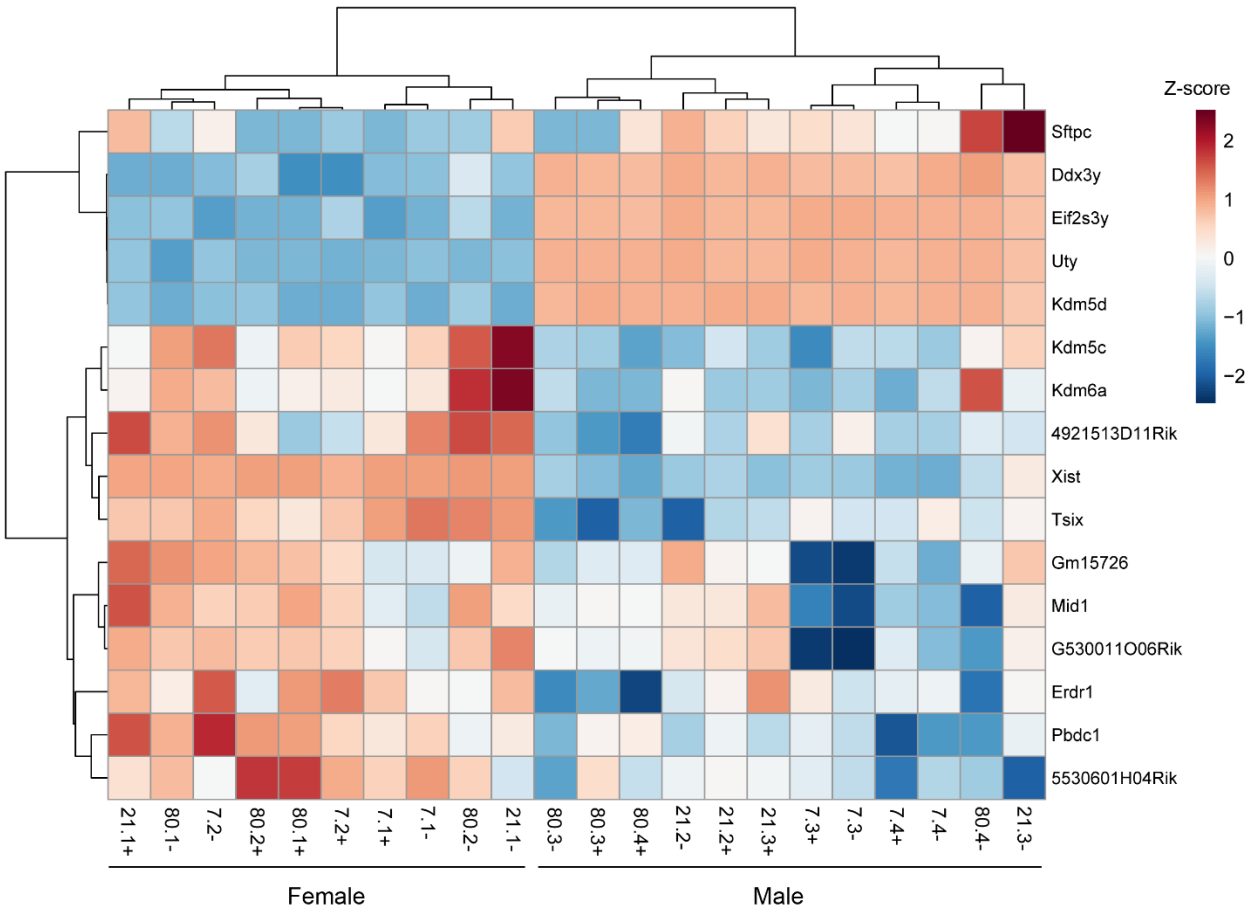

Figure S3: Differential gene expression between male and female mice. Heat map shows differentially expressed genes ($p \leq 0.05$ and at least a two-fold change) between males and females. Includes both GFP+ and GFP- nuclei samples at all ages; for sample ID, first number indicates age of animal, followed by replicate number, followed by +/- to indicate GFP+/GFP-. Gene expression has been z-score normalized and the samples and genes are clustered by correlation distance with complete linkage. None of these genes significantly differentially expressed by males vs. females were enriched in pDyn-GFP nuclei.