

Supplementary Material

Human Skeletal Muscle Tissue Chip Autonomous Payload Reveals Changes in Fiber Type and Metabolic Gene Expression Due to Spaceflight

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Supplementary Table 1. RNA quantification and RNA Integrity Number (RIN) for (A) flight samples and (B) Ground samples.

A. RNA quantity for Flight samples

Chip #	Description	RNA (ng)	RIN
1	Old Estim	1637.7	8.7
2	Old Estim	1216.7	7.1
3	Old Estim	1139.3	7.2
4	Young Estim	1497.8	7.2
5	Young Estim	1434.4	6.8
6	Young Estim	1725.7	9.6
7	Young No Estim	1312.2	7.2
8	Young Estim	1821.8	9.1
9	Old No Estim	1753.2	6.9
10	Old No Estim	2225.6	7.0*
11	Old No Estim	1007.8	7.1*
12	Old No Estim	3505.4	9.0*
13	Young No Estim	1878.7	8.9*
14	Young No Estim	884.1	6.2
15	Young No Estim	1710	7.2*
16	Young No Estim	1188.6	9.0*

B. RNA quantity for Ground samples

Chip #	Description	RNA (ng)	RIN
1	Old No Estim	1395	9.3*
2	Old No Estim	1014	9.3*
3	Old No Estim	1234	9.2*
4	Old No Estim	1650	9.5
5	Young No Estim	1500	9.1*
6	Young No Estim	990	9.1*
7	Young No Estim	1456	9.3*
8	Young No Estim	1284	9.0

*Tissue chips used in the analysis and reported in the communication. Each chip is derived from cells pooled from 5 male donors of the YA cohort or the OS cohort. Estim and No Estim indicates tissue chips that were stimulated over the time course of the experiment vs tissue chips that were not electrically stimulated, respectively.

Supplementary Table 2. Differentially expressed genes in Flight versus Ground young, athletic derived myobundles

Gene	Description	Fold Change	FDR
Upregulated Genes (44)			
<i>AC093525.2</i>	AC093525.2 readthrough	7.164650421	5.21275E-05
<i>CNTNAP2</i>	contactin associated protein 2	5.450281716	0.000617734
<i>GRIA4</i>	glutamate ionotropic receptor AMPA type subunit 4	5.366105495	4.16413E-05
<i>CSF3R</i>	colony stimulating factor 3 receptor	5.048318314	0.040861248
<i>OR51E2</i>	olfactory receptor family 51 subfamily E member 2	4.982648803	0.000195307
<i>DYDC2</i>	DPY30 domain containing 2	4.868519969	0.032258577
<i>CPB1</i>	carboxypeptidase B1	4.157978745	0.025455092
<i>METTL21C</i>	methyltransferase 21C, AARS1 lysine	3.814856526	0.0009246
<i>MCHR1</i>	melanin concentrating hormone receptor 1	3.135941902	2.71353E-07
<i>UMODL1</i>	uromodulin like 1	2.992139178	0.024107585
<i>AC018630.2</i>	AC018630.2 readthrough	2.838326714	0.031842359
<i>ABCG4</i>	ATP binding cassette subfamily G member 4	2.768271531	0.024622362
<i>KCNJ4</i>	potassium inwardly rectifying channel subfamily J member 4	2.597140863	0.000184233
<i>MYRFL</i>	myelin regulatory factor like	2.574460737	1.93839E-06
<i>VWDE</i>	von Willebrand factor D and EGF domains	2.547118719	0.005281277
<i>PFKP</i>	phosphofructokinase, platelet	2.544060278	4.67614E-06
<i>HS6ST3</i>	heparan sulfate 6-O-sulfotransferase 3	2.511162382	0.023621889
<i>COL6A6</i>	collagen type VI alpha 6 chain	2.458016866	0.004006578
<i>TGFBR3L</i>	transforming growth factor beta receptor 3 like	2.42910639	0.024866346
<i>MDF1</i>	MyoD family inhibitor	2.38453661	0.043843058
<i>SERPINA5</i>	serpin family A member 5	2.369500506	0.003012647
<i>PODNL1</i>	podocan like 1	2.324977308	3.5276E-14
<i>FGF12</i>	fibroblast growth factor 12	2.295016809	0.017954825
<i>SLC6A1</i>	solute carrier family 6 member 1	2.287422637	0.007291892
<i>MGP</i>	matrix Gla protein	2.272479259	0.001483125
<i>SCIN</i>	scinderin	2.260979666	0.001273009
<i>ZNF850</i>	zinc finger protein 850	2.251668785	0.002335027
<i>SLCO4A1</i>	solute carrier organic anion transporter family member	2.249036509	1.16401E-07
<i>B3GNT4</i>	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 4	2.244322193	0.028133397

<i>GRM1</i>	glutamate metabotropic receptor 1	2.231068317	3.6986E-05
<i>TTC34</i>	tetratricopeptide repeat domain 34	2.186841093	0.016333667
<i>FAM178B</i>	FAM178B: family with sequence similarity 178 member B	2.183341716	0.001413665
<i>FAM57B</i>	FAM57B: family with sequence similarity 57 member B	2.156290814	7.54584E-15
<i>PALM3</i>	paralemmin 3	2.140786839	0.032658474
<i>TOR4A</i>	torsin family 4 member A	2.127319267	1.89407E-10
<i>YPELA</i>	yipee like 4	2.121103069	0.028785587
<i>CTRL</i>	chymotrypsin like	2.113328046	0.01915699
<i>PLB1</i>	phospholipase B1	2.113285066	0.023783155
<i>CHAC1</i>	ChaC glutathione specific gamma-glutamylcyclotransferase 1	2.113091053	0.001268902
<i>DCDC1</i>	doublecortin domain containing 1	2.101303893	0.029020203
<i>PNLIPRP3</i>	pancreatic lipase related protein 3	2.070574498	0.021410201
<i>C7</i>	complement C7	2.067699643	7.46116E-07
<i>SUSD4</i>	sushi domain containing 4	2.036046359	1.60688E-17
<i>VIL1</i>	villin 1	2.00816373	0.04739108
	Downregulated Genes (110)		
<i>GUCY1A2</i>	guanylate cyclase 1 soluble subunit alpha 2	-2.018093048	1.99411E-10
<i>ZFP36</i>	ZFP36 ring finger protein	-2.018876449	3.01373E-06
<i>EFEMP1</i>	EGF containing fibulin extracellular matrix protein 1	-2.027015633	0.000259005
<i>RIMS3</i>	regulating synaptic membrane exocytosis 3	-2.033630867	0.005667261
<i>PCDHA7</i>	protocadherin alpha 7	-2.034751645	0.028353136
<i>PDE11A</i>	phosphodiesterase 11A	-2.043430345	0.037300533
<i>TMEM178B</i>	transmembrane protein 178B	-2.043941926	5.64787E-36
<i>EGR2</i>	early growth response 2	-2.049376215	6.8619E-05
<i>SPAAR</i>	small regulatory polypeptide of amino acid response	-2.057438265	0.026079467
<i>BIRC3</i>	baculoviral IAP repeat containing 3	-2.065881453	8.43477E-06
<i>NGFR</i>	nerve growth factor receptor	-2.071120425	6.1174E-05
<i>PHACTR3</i>	phosphatase and actin regulator 3	-2.076778696	0.000204077
<i>OAS3</i>	2'-5'-oligoadenylate synthetase 3	-2.087824987	0.000469052
<i>CXCL8</i>	C-X-C motif chemokine ligand 8	-2.125016841	0.016734854
<i>SHISA9</i>	shisa family member 9	-2.126294751	4.22057E-09
<i>JUNB</i>	JunB proto-oncogene, AP-1 transcription factor subunit	-2.127731456	0.000641766
<i>ITGB3</i>	integrin subunit beta 3	-2.131777766	6.56226E-08
<i>FAM43B</i>	family with sequence similarity 43 member B	-2.13361598	0.033720356
<i>CDHR1</i>	cadherin related family member 1	-2.134569371	6.77655E-05

<i>ALS2CR12</i>	ALS2CR12 readthrough	-2.142778176	0.00123712
<i>C11orf96</i>	chromosome 11 open reading frame 96	-2.148358618	0.000238374
<i>CLIC2</i>	chloride intracellular channel 2	-2.156646387	0.036441058
<i>PCDH10</i>	protocadherin 10	-2.162674866	0.00519119
<i>PTX3</i>	pentraxin 3	-2.169064812	3.50516E-05
<i>RCCD1</i>	RCC1 domain containing 1	-2.200601598	0.028435238
<i>AC073896.1</i>	AC073896.1 readthrough	-2.200991483	0.000140442
<i>GCNT1</i>	glucosaminyl (N-acetyl) transferase 1	-2.243308128	0.027705945
<i>GIPC2</i>	GIPC PDZ domain containing family member 2	-2.269410671	0.012464612
<i>TLE2</i>	TLE family member 2, transcriptional corepressor	-2.269757409	0.015884135
<i>OTUD1</i>	OTU deubiquitinase 1	-2.287705143	6.79091E-15
<i>MYH6</i>	myosin heavy chain 6	-2.290182333	8.65344E-07
<i>SBK2</i>	SH3 domain binding kinase family member 2	-2.294608569	0.013172591
<i>CORO2A</i>	coronin 2A	-2.300680372	0.024373908
<i>RANBP17</i>	RAN binding protein 17	-2.314937945	0.040476689
<i>GNRHR</i>	gonadotropin releasing hormone receptor	-2.325688078	0.00815339
<i>C8orf34</i>	chromosome 8 open reading frame 34	-2.33723793	0.028258487
<i>KCNA10</i>	potassium voltage-gated channel subfamily A member 10	-2.343994096	0.02806624
<i>IGSF3</i>	immunoglobulin superfamily member 3	-2.355276695	5.4917E-05
<i>MINOS1-NBL1</i>	MICOS10-NBL1 readthrough	-2.389441362	0.000278839
<i>CSF3</i>	colony stimulating factor 3	-2.438831523	5.61648E-05
<i>CA7</i>	carbonic anhydrase 7	-2.446927221	7.81941E-06
<i>ADGRD1</i>	adhesion G protein-coupled receptor D1	-2.450835201	8.09285E-12
<i>ITIH3</i>	inter-alpha-trypsin inhibitor heavy chain 3	-2.457215484	0.022896877
<i>CTXN3</i>	cortixin 3	-2.459783114	3.56648E-14
<i>EGR1</i>	early growth response 1	-2.485448008	0.000102019
<i>SLC29A4</i>	solute carrier family 29 member 4	-2.501427569	2.20492E-10
<i>ABCA4</i>	ATP binding cassette subfamily A member 4	-2.503758261	0.000635994
<i>TMEM40</i>	transmembrane protein 40	-2.551979434	0.004537744
<i>CDYL2</i>	chromodomain Y like 2	-2.554687625	2.66455E-18
<i>MGAT4C</i>	MGAT4 family member C	-2.557487954	0.033624859
<i>CD24</i>	CD24 molecule	-2.590494325	5.89464E-06
<i>GABRB3</i>	gamma-aminobutyric acid type A receptor subunit beta3	-2.60096351	0.000777337
<i>ADAMTSL4</i>	ADAMTS like 4	-2.621886446	4.6055E-26
<i>DNAH2</i>	dynein axonemal heavy chain 2	-2.625782585	0.000620396

<i>SCN3A</i>	sodium voltage-gated channel alpha subunit 3	-2.634804574	0.000207852
<i>CNTN3</i>	contactin 3	-2.66779935	4.33268E-05
<i>MAS1</i>	MAS1 proto-oncogene, G protein-coupled receptor	-2.694376738	8.96184E-09
<i>PRDM6</i>	PR/SET domain 6	-2.700197512	0.010559536
<i>OR7A5</i>	olfactory receptor family 7 subfamily A member 5	-2.70300847	0.008383645
<i>MTUS2</i>	microtubule associated scaffold protein 2	-2.706234786	1.02358E-05
<i>SLCO2B1</i>	solute carrier organic anion transporter family member 2B1	-2.71005521	0.019104909
<i>CUX2</i>	cut like homeobox 2	-2.715096495	0.012893493
<i>SCN7A</i>	sodium voltage-gated channel alpha subunit 7	-2.72408044	0.013424219
<i>CXCL5</i>	C-X-C motif chemokine ligand 5	-2.779740703	0.017285992
<i>VMO1</i>	vitelline membrane outer layer 1 homolog	-2.798735883	0.016978677
<i>SOCS1</i>	suppressor of cytokine signaling 1	-2.811750603	0.004700644
<i>MMP9</i>	matrix metalloproteinase 9	-2.826698247	0.045205873
<i>FAM19A2</i>	FAM19A2 readthrough	-2.838004149	0.014019371
<i>CACNA1E</i>	calcium voltage-gated channel subunit alpha 1 E	-2.858147793	0.005064968
<i>ONECUT2</i>	one cut homeobox 2	-2.915792192	0.00048654
<i>INSC</i>	INSC spindle orientation adaptor protein	-2.916515798	0.014456608
<i>DGAT2</i>	diacylglycerol O-acyltransferase 2	-2.919201125	0.045426703
<i>DMBT1</i>	deleted in malignant brain tumors 1	-2.94457787	2.44486E-05
<i>FOS</i>	Fos proto-oncogene, AP-1 transcription factor subunit	-2.991999455	0.000247171
<i>CXCL13</i>	C-X-C motif chemokine ligand 13	-3.067756845	3.47741E-16
<i>ADRB2</i>	adrenoceptor beta 2	-3.096994028	7.76539E-19
<i>SERPINB7</i>	serpin family B member 7	-3.187850298	0.00519119
<i>FLRT1</i>	fibronectin leucine rich transmembrane protein 1	-3.270832353	0.019496782
<i>PTPRC</i>	protein tyrosine phosphatase receptor type C	-3.287150062	0.006176527
<i>RELN</i>	reelin	-3.319118652	0.000292176
<i>PK4</i>	pyruvate dehydrogenase kinase 4	-3.325146285	3.85134E-05
<i>FOSB</i>	FosB proto-oncogene, AP-1 transcription factor subunit	-3.375890553	7.71904E-07
<i>ANO2</i>	anoctamin 2	-3.446938388	0.003711354
<i>ESM1</i>	endothelial cell specific molecule 1	-3.500513259	0.007727148
<i>KISS1</i>	KiSS-1 metastasis suppressor	-3.600023239	0.0001874
<i>SSTR2</i>	somatostatin receptor 2	-3.619293532	0.009280342
<i>TMEM110-MUSTN1</i>	TMEM110-MUSTN1 readthrough	-3.648751254	7.35509E-05
<i>SMTNL1</i>	smoothelin like 1	-3.6934315	1.00875E-06
<i>MYL10</i>	myosin light chain 10	-3.95865901	9.39304E-08

<i>PDZD2</i>	PDZ domain containing 2	-3.98994048	0.00136236
<i>SCARA5</i>	scavenger receptor class A member 5	-4.009235804	7.62091E-21
<i>PVALB</i>	parvalbumin	-4.0198962	0.000257454
<i>SYT1</i>	synaptotagmin 1	-4.034078439	0.000140442
<i>PSG11</i>	pregnancy specific beta-1-glycoprotein 11	-4.113972439	0.012018776
<i>CUZD1</i>	CUB and zona pellucida like domains 1	-4.116254828	1.61224E-05
<i>DIRAS2</i>	DIRAS family GTPase 2	-4.118507424	0.007374237
<i>TM4SF20</i>	transmembrane 4 L six family member 20	-4.166515956	0.000391648
<i>DEFB103B</i>	defensin beta 103B	-4.214503417	0.000139906
<i>KCNA2</i>	potassium voltage-gated channel subfamily A member 2	-4.217102228	0.006392639
<i>AC013489.1</i>	AC013489.1 readthrough	-4.29376469	0.032170351
<i>STEAP4</i>	STEAP4 metalloreductase	-4.403227272	0.002524526
<i>RPTN</i>	repetin	-4.470907609	8.15675E-06
<i>KPRP</i>	keratinocyte proline rich protein	-4.526968504	0.000157524
<i>LY75-CD302</i>	LY75-CD302 readthrough	-4.591585426	0.027003205
<i>CXCL6</i>	C-X-C motif chemokine ligand 6	-4.602979064	0.006890469
<i>CISH</i>	cytokine inducible SH2 containing protein	-4.645653524	0.001169639
<i>GCNT3</i>	glucosaminyl (N-acetyl) transferase 3, mucin type	-4.665883943	3.33171E-15
<i>SERPINB2</i>	serpin family B member 2	-5.469421134	0.001240009
<i>SULT1A4</i>	sulfotransferase family 1A member 4	-5.943844153	0.007648353
<i>PSG1</i>	pregnancy specific beta-1-glycoprotein 1	-6.233947331	0.001443649

Supplementary Table 3. Differentially expressed genes in Flight versus Ground old, sedentary derived myobundles

<i>Gene</i>	Description	Fold Change	FDR
	Upregulated Genes (99)		
<i>HIST2H2AA3</i>	HIST2H2AA3 readthrough	9.996972635	0.014365614
<i>AC048338.1</i>	AC048338.1 readthrough	9.896124868	6.53643E-06
<i>CSF3R</i>	colony stimulating factor 3 receptor	7.336449293	0.000980146
<i>HBA2</i>	hemoglobin subunit alpha 2	6.629040764	0.005256727
<i>AP000350.4</i>	AP000350.4 readthrough	6.200975095	0.008942407
<i>SLC14A1</i>	solute carrier family 14 member 1 (Kidd blood group)	5.833977572	0.001174276
<i>KRT19</i>	keratin 19	5.284363775	0.00127304
<i>RSAD2</i>	radical S-adenosyl methionine domain containing 2	5.057491019	0.000376949
<i>GAL3ST1</i>	galactose-3-O-sulfotransferase 1	4.440323571	0.000592674
<i>CPB1</i>	carboxypeptidase B1	4.384920788	0.000258081
<i>FSCN3</i>	fascin actin-bundling protein 3	4.193087119	0.000633467
<i>KRT8</i>	keratin 8	4.151324259	0.015616573
<i>ENO4</i>	enolase 4	4.085678152	0.008847841
<i>METTL21C</i>	methyltransferase 21C, AARS1 lysine	3.965837441	0.000487923
<i>ARHGAP36</i>	Rho GTPase activating protein 36	3.916108217	0.030072269
<i>RORC</i>	RAR related orphan receptor C	3.893111603	4.48508E-38
<i>KCNJ3</i>	potassium inwardly rectifying channel subfamily J member 3	3.784502622	0.00083577
<i>ST20-MTHFS</i>	ST20-MTHFS readthrough	3.762067682	0.001953065
<i>NOS1</i>	nitric oxide synthase 1	3.703729233	3.58378E-15
<i>FAM57B</i>	FAM57B readthrough	3.703378318	9.72522E-45
<i>SERPINA5</i>	serpin family A member 5	3.679110525	2.27285E-07
<i>LCPI</i>	lymphocyte cytosolic protein 1	3.671761138	0.011029286
<i>CNTNAP2</i>	contactin associated protein 2	3.538737469	0.003434529
<i>OR51E2</i>	olfactory receptor family 51 subfamily E member 2	3.44537276	0.000345925
<i>IRF4</i>	interferon regulatory factor 4	3.417250424	0.013594084
<i>HSD11B2</i>	hydroxysteroid 11-beta dehydrogenase 2	3.26539635	5.30574E-09
<i>FAM83E</i>	family with sequence similarity 83 member E	3.187961906	0.044733397
<i>MTCPI</i>	mature T cell proliferation 1	3.178835087	0.014035233
<i>LYZ</i>	lysozyme	3.149502048	0.002253136
<i>SLCO4A1</i>	solute carrier organic anion transporter family member 4A1	3.132334346	2.38179E-14
<i>MYRFL</i>	myelin regulatory factor like	3.081932779	6.03269E-09

<i>CH25H</i>	cholesterol 25-hydroxylase	3.07467968	0.006172311
<i>ADAM8</i>	ADAM metallopeptidase domain 8	3.026603305	0.001069424
<i>CSKMT</i>	citrate synthase lysine methyltransferase	2.984105134	1.48198E-08
<i>KIAA0040</i>	KIAA0040 readthrough	2.897171856	0.000153288
<i>RAB33A</i>	RAB33A, member RAS oncogene family	2.880591264	0.020934461
<i>CORO1A</i>	coronin 1A	2.879991295	0.039013179
<i>SLC37A2</i>	solute carrier family 37 member 2	2.876235031	0.031833815
<i>TRIB3</i>	tribbles pseudokinase 3	2.872474462	8.90624E-18
<i>LLGL2</i>	LLGL scribble cell polarity complex component 2	2.844440683	1.44856E-09
<i>SLC6A9</i>	solute carrier family 6 member 9	2.820442933	2.7412E-05
<i>C2CD4A</i>	C2 calcium dependent domain containing 4A	2.802341488	1.80206E-08
<i>CHAD</i>	chondroadherin	2.793136991	0.002692199
<i>CHAC1</i>	ChaC glutathione specific gamma-glutamylcyclotransferase 1	2.791954301	1.58653E-06
<i>LINGO4</i>	leucine rich repeat and Ig domain containing 4	2.77428638	0.02268445
<i>NPBWR1</i>	neuropeptides B and W receptor 1	2.770595739	0.000171063
<i>SCUBE2</i>	signal peptide, CUB domain and EGF like domain containing 2	2.725747424	1.63132E-21
<i>C4orf17</i>	chromosome 4 open reading frame 17	2.715711659	0.030599871
<i>PCK2</i>	phosphoenolpyruvate carboxykinase 2, mitochondrial	2.688584044	2.39918E-19
<i>MX1</i>	MX dynamin like GTPase 1	2.627265865	0.000312272
<i>SLC9A3</i>	solute carrier family 9 member A3	2.624480958	0.00451659
<i>ABCB4</i>	ATP binding cassette subfamily B member 4	2.613250985	0.022695037
<i>CA14</i>	carbonic anhydrase 14	2.600229786	0.00088289
<i>PCLAF</i>	PCNA clamp associated factor	2.600007731	0.013165177
<i>PHGDH</i>	phosphoglycerate dehydrogenase	2.597687813	9.60059E-16
<i>PLCB2</i>	phospholipase C beta 2	2.587428562	5.05261E-05
<i>KRT18</i>	keratin 18	2.556450727	1.31829E-05
<i>ADRA2A</i>	adrenoceptor alpha 2A	2.554008547	7.19851E-13
<i>SLC6A1</i>	solute carrier family 6 member 1	2.545584324	0.001193861
<i>GCOM1</i>	GCOM1, MYZAP-POLR2M combined locus	2.543365656	0.009504753
<i>APLN</i>	apelin	2.485238218	0.003614168
<i>MMP9</i>	matrix metallopeptidase 9	2.480190644	0.041806699
<i>GRM1</i>	glutamate metabotropic receptor 1	2.451745383	5.76266E-07
<i>ELN</i>	elastin	2.442668494	1.42308E-18
<i>TRPM8</i>	transient receptor potential cation channel subfamily M member 8	2.43841871	1.609E-05
<i>KCNRG</i>	potassium channel regulator	2.424743962	0.005477797

<i>PLIN4</i>	perilipin 4	2.418158315	2.50827E-15
<i>ABCG1</i>	ATP binding cassette subfamily G member 1	2.409928463	9.60356E-26
<i>ASB10</i>	ankyrin repeat and SOCS box containing 10	2.409437385	2.6281E-06
<i>PERM1</i>	PPARGC1 and ESRR induced regulator, muscle 1	2.393578989	3.37929E-09
<i>GLDN</i>	gliomedin	2.390485559	4.17275E-05
<i>BRICD5</i>	BRICHOS domain containing 5	2.37946023	0.017334931
<i>OAS1</i>	2'-5'-oligoadenylate synthetase 1	2.358644057	0.017324701
<i>NDRG4</i>	NDRG family member 4	2.348510796	2.53496E-12
<i>IFITM1</i>	interferon induced transmembrane protein 1	2.341173199	0.000385455
<i>TFCP2L1</i>	transcription factor CP2 like 1	2.330740277	0.043211995
<i>FHAD1</i>	forkhead associated phosphopeptide binding domain 1	2.310468721	0.003984946
<i>NOXO1</i>	NADPH oxidase organizer 1	2.303047422	0.000168865
<i>FAM178B</i>	family with sequence similarity 178 member B	2.296591338	0.000829266
<i>THRSP</i>	thyroid hormone responsive	2.291401212	0.004577044
<i>MCHR1</i>	melanin concentrating hormone receptor 1	2.280855022	0.00021079
<i>EXOC3L1</i>	exocyst complex component 3 like 1	2.237784772	1.18055E-05
<i>POU2F2</i>	POU class 2 homeobox 2	2.225137741	0.038942049
<i>NRN1L</i>	neuritin 1 like	2.208437259	0.000551956
<i>ARG2</i>	arginase 2	2.181974414	6.02253E-07
<i>HSPA2</i>	heat shock protein family A (Hsp70) member 2	2.181613481	0.001367034
<i>VIL1</i>	villin 1	2.154467686	0.027073905
<i>RTN4RL2</i>	reticulon 4 receptor like 2	2.143646973	6.39305E-10
<i>EGFL7</i>	EGF like domain multiple 7	2.130354194	7.92453E-25
<i>RASGRP2</i>	RAS guanyl releasing protein 2	2.105147255	1.17602E-10
<i>PFKP</i>	phosphofructokinase, platelet	2.079083902	0.000185431
<i>CD101</i>	CD101 molecule	2.049526837	3.26453E-05
<i>FAM129A</i>	FAM129A readthrough	2.037754959	1.02031E-21
<i>KCNS3</i>	potassium voltage-gated channel modifier subfamily S member 3	2.026461785	5.67255E-14
<i>TOR4A</i>	torsin family 4 member A	2.02357543	5.99937E-11
<i>CCDC28B</i>	coiled-coil domain containing 28B	2.020495327	1.16227E-13
<i>PRODH</i>	proline dehydrogenase 1	2.020243469	4.24532E-11
<i>COL15A1</i>	collagen type XV alpha 1 chain	2.014387784	8.45469E-18
<i>ADM2</i>	adrenomedullin 2	2.013952235	0.004454598
<i>CBS</i>	cystathionine beta-synthase	2.002847428	4.34413E-13
Downregulated Genes (153)			

<i>PAPPA</i>	pappalysin 1	-2.001698621	1.68884E-05
<i>MMP19</i>	matrix metalloproteinase 19	-2.004168809	0.00024325
<i>CAPN9</i>	calpain 9	-2.00701002	0.001289031
<i>IDI</i>	inhibitor of DNA binding 1	-2.008436028	0.001189951
<i>EFEMP1</i>	EGF containing fibulin extracellular matrix protein 1	-2.01222761	8.39305E-05
<i>SERPINE1</i>	serpin family E member 1	-2.012798599	0.007341419
<i>MID1</i>	midline 1	-2.014133958	2.71322E-08
<i>ADGRV1</i>	adhesion G protein-coupled receptor V1	-2.016315779	8.46852E-06
<i>NCALD</i>	neurocalcin delta	-2.024698413	3.27892E-13
<i>MAPK4</i>	mitogen-activated protein kinase 4	-2.031338479	5.82168E-08
<i>PLCXD2</i>	phosphatidylinositol specific phospholipase C X domain containing 2	-2.048349705	0.001047584
<i>MINAR1</i>	membrane integral NOTCH2 associated receptor 1	-2.052158468	1.47777E-18
<i>DLK1</i>	delta like non-canonical Notch ligand 1	-2.06513025	0.008519671
<i>SSC5D</i>	scavenger receptor cysteine rich family member with 5 domains	-2.065761861	5.89858E-06
<i>L3MBTL4</i>	L3MBTL histone methyl-lysine binding protein 4	-2.075094071	0.016297521
<i>EXPH5</i>	exophilin 5	-2.088316686	0.001693647
<i>NKD1</i>	NKD inhibitor of WNT signaling pathway 1	-2.089314247	4.99209E-06
<i>CNTN3</i>	contactin 3	-2.101849509	0.001064981
<i>MAP6</i>	microtubule associated protein 6	-2.105741108	0.000477822
<i>PALM2</i>	paralemmin 2	-2.106223896	1.2873E-10
<i>PLGLB1</i>	plasminogen like B1	-2.110172055	0.023820057
<i>CPM</i>	carboxypeptidase M	-2.118777225	0.018017253
<i>OTUD1</i>	OTU deubiquitinase 1	-2.130851869	1.51378E-13
<i>FAM184B</i>	family with sequence similarity 184 member B	-2.132789747	5.04975E-07
<i>CHIT1</i>	chitinase 1	-2.143020897	0.000140463
<i>ADRB2</i>	adrenoceptor beta 2	-2.152287789	2.9653E-10
<i>PAMR1</i>	peptidase domain containing associated with muscle regeneration 1	-2.168966168	5.07082E-10
<i>CMTM5</i>	CKLF like MARVEL transmembrane domain containing 5	-2.181187893	0.000343903
<i>GUCY1A2</i>	guanylate cyclase 1 soluble subunit alpha 2	-2.182501382	1.84819E-14
<i>CXCL5</i>	C-X-C motif chemokine ligand 5	-2.185653502	0.043003003
<i>PRR18</i>	proline rich 18	-2.187533016	0.01263233
<i>SAMD13</i>	sterile alpha motif domain containing 13	-2.195411319	0.014011783
<i>SLC6A17</i>	solute carrier family 6 member 17	-2.199972562	1.59345E-05
<i>PPP1R1A</i>	protein phosphatase 1 regulatory inhibitor subunit 1A	-2.203083641	8.02612E-06
<i>MFAP5</i>	microfibril associated protein 5	-2.20389193	3.08199E-05

<i>POU5F1B</i>	POU class 5 homeobox 1B	-2.20902513	1.19406E-07
<i>ADAMTSL4</i>	ADAMTS like 4	-2.212038108	2.71408E-19
<i>C19orf81</i>	chromosome 19 open reading frame 81	-2.223905987	0.032471623
<i>RNF165</i>	ring finger protein 165	-2.226441009	0.045548378
<i>AGXT</i>	alanine--glyoxylate aminotransferase	-2.227146998	0.011745612
<i>KCNE5</i>	potassium voltage-gated channel subfamily E regulatory subunit 5	-2.232802059	1.68087E-09
<i>ACTN3</i>	actinin alpha 3	-2.236539699	1.94493E-05
<i>LGII</i>	leucine rich glioma inactivated 1	-2.244541331	0.00129478
<i>PCDHB3</i>	protocadherin beta 3	-2.248507738	0.041636754
<i>PPP1R27</i>	protein phosphatase 1 regulatory subunit 27	-2.250189692	2.95039E-07
<i>P2RX1</i>	purinergic receptor P2X 1	-2.256160133	9.65365E-06
<i>TMEM217</i>	transmembrane protein 217	-2.265371971	0.043131825
<i>C8orf34</i>	chromosome 8 open reading frame 34	-2.267123331	0.020709145
<i>SERPINB7</i>	serpin family B member 7	-2.267475774	0.031491506
<i>SPAG6</i>	sperm associated antigen 6	-2.271276579	0.000294406
<i>CDH8</i>	cadherin 8	-2.275398912	1.76668E-07
<i>MGAM</i>	maltase-glucoamylase	-2.289340221	0.002179971
<i>AC008073.3</i>	AC008073.3 readthrough	-2.29185413	0.023138575
<i>SLC38A4</i>	solute carrier family 38 member 4	-2.29421038	0.004517197
<i>CPXM2</i>	carboxypeptidase X, M14 family member 2	-2.295883371	0.036290694
<i>CDK5R2</i>	cyclin dependent kinase 5 regulatory subunit 2	-2.299826607	0.016450514
<i>AMPH</i>	amphiphysin	-2.306415442	0.000281947
<i>CDHR1</i>	cadherin related family member 1	-2.307338568	1.08457E-05
<i>CDH22</i>	cadherin 22	-2.316198091	0.025658864
<i>ECM2</i>	extracellular matrix protein 2	-2.319226085	0.000944036
<i>OCA2</i>	OCA2 melanosomal transmembrane protein	-2.320966428	0.046970969
<i>MMP13</i>	matrix metalloproteinase 13	-2.322828809	0.001946904
<i>PLEKHG7</i>	pleckstrin homology and RhoGEF domain containing G7	-2.328091062	0.019518382
<i>PIP5K1B</i>	phosphatidylinositol-4-phosphate 5-kinase type 1 beta	-2.32866678	1.58839E-09
<i>ITGA9</i>	integrin subunit alpha 9	-2.330332409	3.17321E-05
<i>ILDR2</i>	immunoglobulin like domain containing receptor 2	-2.345924597	0.005777283
<i>SCN2A</i>	sodium voltage-gated channel alpha subunit 2	-2.351689839	0.01612589
<i>IL32</i>	interleukin 32	-2.356595964	0.00101918
<i>HSD11B1</i>	hydroxysteroid 11-beta dehydrogenase 1	-2.362218595	0.000616679
<i>NPAS1</i>	neuronal PAS domain protein 1	-2.38509115	2.14913E-09
<i>CELF4</i>	CUGBP Elav-like family member 4	-2.397723746	0.01864044

<i>SDK2</i>	sidekick cell adhesion molecule 2	-2.414618146	5.00587E-30
<i>FAM43B</i>	family with sequence similarity 43 member B	-2.417441818	0.005734886
<i>RELN</i>	reelin	-2.443817102	0.003483523
<i>BTBD8</i>	BTB domain containing 8	-2.475392377	0.000984211
<i>LONRF2</i>	LON peptidase N-terminal domain and ring finger 2	-2.478477798	2.16136E-11
<i>SCN7A</i>	sodium voltage-gated channel alpha subunit 7	-2.482115699	0.005655595
<i>ADAP1</i>	ArfGAP with dual PH domains 1	-2.490141884	0.000516603
<i>AIRE</i>	autoimmune regulator	-2.495767873	1.89836E-06
<i>DPYSL5</i>	dihydropyrimidinase like 5	-2.509677934	1.68175E-15
<i>NHLH2</i>	nescient helix-loop-helix 2	-2.515217017	9.32663E-30
<i>GNAT1</i>	G protein subunit alpha transducin 1	-2.526506819	0.029138282
<i>UGT2B4</i>	UDP glucuronosyltransferase family 2 member B4	-2.571625403	3.85685E-06
<i>TMEM178B</i>	transmembrane protein 178B	-2.59172763	1.17119E-59
<i>GJA5</i>	gap junction protein alpha 5	-2.609205441	1.64855E-08
<i>AFF3</i>	ALF transcription elongation factor 3	-2.652820225	0.047420947
<i>MYH6</i>	myosin heavy chain 6	-2.660505638	3.32316E-09
<i>MYH1</i>	myosin heavy chain 1	-2.667226287	4.28409E-13
<i>INA</i>	internexin neuronal intermediate filament protein alpha	-2.668632236	0.010260118
<i>NKAIN4</i>	sodium/potassium transporting ATPase interacting 4	-2.683826755	0.034810829
<i>DCX</i>	doublecortin	-2.709482013	3.53112E-08
<i>DIRAS2</i>	DIRAS family GTPase 2	-2.731609283	0.0080137
<i>BEGAIN</i>	brain enriched guanylate kinase associated	-2.737767166	0.037039297
<i>ZNF781</i>	zinc finger protein 781	-2.750962443	0.041433385
<i>SCARA5</i>	scavenger receptor class A member 5	-2.768009597	4.39118E-10
<i>GALR1</i>	galanin receptor 1	-2.801132683	0.037139691
<i>PDK4</i>	pyruvate dehydrogenase kinase 4	-2.831019397	0.00051737
<i>PDZD2</i>	PDZ domain containing 2	-2.850566244	0.010004297
<i>NGFR</i>	nerve growth factor receptor	-2.879977093	3.16514E-13
<i>NRARP</i>	NOTCH regulated ankyrin repeat protein	-2.967003151	0.034104813
<i>RPTN</i>	repetin	-2.975318581	0.00362023
<i>AC010422.3</i>	AC010422.3 readthrough	-3.034696001	0.033209818
<i>ARC</i>	activity regulated cytoskeleton associated protein	-3.043765555	0.001840919
<i>GDF6</i>	growth differentiation factor 6	-3.04822861	9.08586E-15
<i>SAMD15</i>	sterile alpha motif domain containing 15	-3.05838817	0.023021402
<i>C12orf42</i>	chromosome 12 open reading frame 42	-3.098787019	0.005038486
<i>TBC1D30</i>	TBC1 domain family member 30	-3.116741964	1.85836E-06

<i>CIQL4</i>	complement C1q like 4	-3.122095899	0.005801968
<i>RYR3</i>	ryanodine receptor 3	-3.152862339	6.23527E-06
<i>CACNA1E</i>	calcium voltage-gated channel subunit alpha1 E	-3.154784862	0.001024993
<i>KCNQ1</i>	potassium voltage-gated channel subfamily Q member 1	-3.172358287	1.70395E-07
<i>GSG1</i>	germ cell associated 1	-3.173827976	5.15447E-05
<i>ESRP1</i>	epithelial splicing regulatory protein 1	-3.184071355	0.02214051
<i>GPBAR1</i>	G protein-coupled bile acid receptor 1	-3.196218517	0.044440742
<i>IGF1</i>	insulin like growth factor 1	-3.212495355	0.007945092
<i>FREM2</i>	FRAS1 related extracellular matrix 2	-3.263565817	2.07025E-54
<i>LRRIQ1</i>	leucine rich repeats and IQ motif containing 1	-3.297080775	0.010536406
<i>CD24</i>	CD24 molecule	-3.306524939	1.5196E-09
<i>RASSF2</i>	Ras association domain family member 2	-3.341046201	3.70519E-06
<i>ZNF730</i>	zinc finger protein 730	-3.355318377	0.031543199
<i>PSG11</i>	pregnancy specific beta-1-glycoprotein 11	-3.396968389	0.022314738
<i>DRP2</i>	dystrophin related protein 2	-3.39969905	4.87849E-36
<i>CCDC170</i>	coiled-coil domain containing 170	-3.419722597	0.0184137
<i>STMN2</i>	stathmin 2	-3.438255383	7.39406E-11
<i>CUX2</i>	cut like homeobox 2	-3.474253	0.00101918
<i>MYH2</i>	myosin heavy chain 2	-3.493656227	6.68099E-24
<i>CXCL13</i>	C-X-C motif chemokine ligand 13	-3.680230955	4.13211E-20
<i>MALL</i>	mal, T cell differentiation protein like	-3.684467646	1.07442E-08
<i>NTF3</i>	neurotrophin 3	-3.705363066	0.026628825
<i>KPRP</i>	keratinocyte proline rich protein	-3.718420971	3.33559E-05
<i>SCN3A</i>	sodium voltage-gated channel alpha subunit 3	-3.764869992	1.88268E-08
<i>GCNT3</i>	glucosaminyl (N-acetyl) transferase 3, mucin type	-3.826071587	1.63143E-10
<i>SERPINB2</i>	serpin family B member 2	-3.917210704	0.001438626
<i>SERPINB12</i>	serpin family B member 12	-3.936814001	0.000239984
<i>CSMD1</i>	CUB and Sushi multiple domains 1	-3.94524382	1.15316E-09
<i>ADGRD1</i>	adhesion G protein-coupled receptor D1	-3.968823097	6.57796E-28
<i>ADGRB3</i>	adhesion G protein-coupled receptor B3	-3.98337514	0.004901084
<i>NLRP10</i>	NLR family pyrin domain containing 10	-4.157116629	0.005115268
<i>INSC</i>	INSC spindle orientation adaptor protein	-4.194969484	0.001832198
<i>MAS1</i>	MAS1 proto-oncogene, G protein-coupled receptor	-4.374162588	6.0226E-22
<i>CHRM2</i>	cholinergic receptor muscarinic 2	-4.403697891	0.017115536
<i>MYL10</i>	myosin light chain 10	-4.419396312	1.17555E-07
<i>KISS1</i>	KiSS-1 metastasis suppressor	-4.423496137	2.69415E-07

<i>CTXN3</i>	cortixin 3	-4.507775368	2.69496E-35
<i>KCNA10</i>	potassium voltage-gated channel subfamily A member 10	-4.584129996	1.61719E-05
<i>SAA1</i>	serum amyloid A1	-4.86295248	0.021436073
<i>TM4SF20</i>	transmembrane 4 L six family member 20	-5.05040186	0.000332029
<i>SLC5A1</i>	solute carrier family 5 member 1	-5.151840374	0.004103828
<i>AL049839.2</i>	AL049839.2 readthrough	-5.545299993	0.040980606
<i>AC018512.1</i>	AC018512.1 readthrough	-5.712811369	0.003424014
<i>PVALB</i>	parvalbumin	-6.963866832	2.01617E-05
<i>CEMP1</i>	cementum protein 1	-7.01121302	0.034021725
<i>DEFB103B</i>	defensin beta 103B	-7.244418203	3.71178E-07

Supplementary Table 4. Gene Ontology enrichment analysis in Flight versus Ground young, athletic derived myobundle

GO term categories	Term	Count	p-value	Genes
Cellular Component	GO:0016021; integral component of plasma membrane	51	0.0043	<i>GABRB3, CNTNAP2, CSF3R, STEAP4, MYRFL, SLC6A1, CXCL13, PLB1, GRM1, HS6ST3, SLCO2B1, GNRHR, DGAT2, TMEM178B, TMEM40, SHISA9, ANO2, SLCO4A1, CDHR1, OAS3, B3GNT4, OR7A5, SLC29A4, KCNA10, CTXN3, ABCG4, TOR4A, IGSF3, PCDH10, KCNA2, GCNT1, ADRB2, MCHR1, CUZD1, TGFBR3L, FLRT1, SUS4, TM4SF20, GCNT3, SCN3A, GRIA4, KCNJ4, NGFR, SYT1, OR51E2, ABCA4, MGAT4C, MAS1, ADGRD1, PTPRC, UMODL1.</i>
	GO:0005886; plasma membrane	48	0.014	<i>GABRB3, STEAP4, CSF3R, PCDH10, ITGB3, PALM3, KCNA2, DIRAS2, ADRB2, SLC6A1, CACNA1E, MCHR1, PLB1, GRM1, RELN, PDZD2, SLCO2B1, FLRT1, INSC, TM4SF20, GNRHR, CLIC2, SCN3A, PCDHA7, GRIA4, KCNJ4, NGFR, SERPINB2, CISH, SYT1, SCARA5, OR51E2, PSG1, SSTR2, ANO2, VIL1, MAS1, ADGRD1, PTPRC, SLCO4A1, OAS3, CDHR1, OR7A5, SLC29A4, CNTN3, KCNA10, RCCD1, ABCG4.</i>
	GO:0016020; membrane	37	0.012	<i>TOR4A, CNTNAP2, STEAP4, IGSF3, KCNA2, GCNT1, DIRAS2, ADRB2, SLC6A1, CUZD1, HS6ST3, SERPINA5, TGFBR3L, RIMS3, DMBT1, SLCO2B1, SUS4, TM4SF20, GNRHR, GCNT3, CLIC2, NGFR, DGAT2, TMEM178B, ABCA4, TMEM40, FOS, ADGRD1, PTPRC, OAS3, CDHR1, OR7A5, KCNA10, CTXN3, CD24, ABCG4, PFKP.</i>
	GO:0005576; extracellular region	30	0.00012	<i>TOR4A, DEFB103B, CXCL6, CSF3, ITIH3, CSF3R, CXCL8, PSG11, CXCL13, CXCL5, SERPINA5, ESM1, RELN, EFEMP1, PDZD2, C7, DMBT1, ADAMTSL4, SUS4, RPTN, NGFR, SERPINB2, PSG1, VWDE, MMP9, KISS1, MGP, CNTN3, COL6A6, PTX3.</i>
	GO:0005615; extracellular space	28	0.00015	<i>DEFB103B, CXCL6, CSF3, CPB1, CXCL8, GCNT1, CXCL13, CXCL5, SERPINA5, VMO1, TGFBR3L, PODNL1, RELN, EFEMP1, C7, DMBT1, FLRT1, TLE2, SERPINB2, SERPINB7, MMP9, KISS1, CTRL, OAS3, PTX3, FGF12, PNLIPRP3, UMODL1.</i>
	GO:0005887; integral component of plasma membrane	22	0.00043	<i>GABRB3, NGFR, CSF3R, SCARA5, PCDH10, ITGB3, ABCA4, KCNA2, ADRB2, SSTR2, SLC6A1, MCHR1, GRM1, MAS1, ADGRD1, PTPRC, SLCO4A1, SLCO2B1, CDHR1, FLRT1, GNRHR, PCDHA7.</i>
Molecular Function	GO:0042802; identical protein binding	18	0.00095	<i>GABRB3, SYT1, ITGB3, PRDM6, FOS, ADRB2, SLC6A1, MMP9, CXCL5, VIL1, ADAMTSL4, GIPC2, PTX3, ABCG4, PCDHA7, PVALB, MDFI, PFKP</i>
	GO:0005509; calcium ion binding	14	0.0011	<i>SYT1, PCDH10, VWDE, CACNA1E, VIL1, SCIN, EFEMP1, CDHR1, MGP, MYL10, PCDHA7, RPTN, PVALB, UMODL1.</i>

Supplementary Table 5. Gene Ontology enrichment analysis in Flight versus Ground old, sedentary derived myobundles

GO term categories	Term	Count	p-value	Genes
Cellular Component	GO:0005886; plasma membrane	93	7.86E-07	<i>CHRM2, CPM, IFITM1, CSF3R, GAL3ST1, SERPINE1, DIRAS2, SLC6A1, GLDN, GRM1, CDH8, RYR3, RASSF2, GJA5, SLCO2B1, TBC1D30, INSC, GSG1, GNAT1, NOS1, CA14, MINAR1, SERPINB2, ADGRV1, SCARAS, ACTN3, GPBAR1, RTN4RL2, RAB33A, ADRA2A, SLC9A3, ARC, NRN1L, ADGRB3, SLCO4A1, CDHR1, KCNQ1, MALL, PLIN4, AMPH, KCNA10, TRIB3, ADAM8, LCP1, PLCB2, FREM2, ABCG1, CD101, LLGL2, ITGA9, SERPINB12, KCNE5, ABCB4, NOXO1, ADRB2, SLC5A1, RASGRP2, CACNA1E, CORO1A, MCHR1, NKD1, DRP2, NKAIN4, RELN, PDZD2, NPBWR1, GALR1, CDH22, TM4SF20, PIP5K1B, TRPM8, SCN3A, KCNJ3, SLC38A4, SLC14A1, NGFR, MGAM, OR51E2, MX1, HSPA2, ADAP1, VIL1, MAS1, ADGRD1, KRT19, SLC6A9, P2RX1, KCNS3, CNTN3, PCDHB3, SCN2A, SDK2, CDK5R2.</i>
	GO:0016021; integral component of membrane	79	0.0039	<i>CHRM2, CNTNAP2, CPM, IFITM1, CSF3R, GAL3ST1, MYRFL, SLC6A1, CXCL13, GLDN, GRM1, CDH8, RYR3, GJA5, SLCO2B1, GSG1, CA14, BRICD5, MINAR1, ADGRV1, TMEM178B, GPBAR1, MID1, SLC9A3, CH25H, MMP13, ADGRB3, SLCO4A1, CDHR1, KCNQ1, PAPP, TMEM217, MALL, UGT2B4, KCNA10, ADAM8, CTXN3, FREM2, ABCG1, CD101, TOR4A, OCA2, COL15A1, NDRG4, KCNE5, ABCB4, ILDR2, ADRB2, SLC5A1, MCHR1, KIAA0040, HSD11B1, HSD11B2, NKAIN4, NPBWR1, GALR1, CDH22, TM4SF20, GCNT3, TRPM8, SCN3A, CSMD1, KCNJ3, SLC38A4, SLC14A1, NGFR, MGAM, LINGO4, SLC37A2, CMTM5, OR51E2, DLK1, MAS1, ADGRD1, P2RX1, KCNS3, PCDHB3, SCN2A, SDK2.</i>
	GO:0016020; membrane	63	5.95E-04	<i>CHRM2, CNTNAP2, IFITM1, GAL3ST1, DIRAS2, STMN2, TFCP2L1, SLC6A1, RYR3, SERPINA5, SLCO2B1, GNAT1, CA14, BRICD5, ADGRV1, SLC6A17, TMEM178B, HBA2, CH25H, NRN1L, OAS1, ADGRB3, IRF4, CDHR1, KCNQ1, TMEM217, MALL, KCNA10, CTXN3, ABCG1, CD101, TOR4A, COL15A1, ABCB4, ILDR2, ADRB2, SLC5A1, CORO1A, KIAA0040, BEGAIN, HSD11B1, NPBWR1, GALR1, TM4SF20, GCNT3, CSMD1, IL32, NGFR, MGAM, LINGO4, CMTM5, MX1, SSC5D, HSPA2, DLK1, ADGRD1, SLC6A9, KCNS3, PCDHB3, SCN2A, CD24, PFKP, CDK5R2.</i>
	GO:0005576; extracellular region	45	0.000031	<i>TOR4A, DEFB103B, CPM, COL15A1, CSF3R, ECM2, PLGLB1, ELN, SERPINE1, PSG11, CXCL13, CXCL5, SERPINA5, SCUBE2, RELN, EFEMP1, PDZD2, ADAMTSL4, NTF3, PAMR1, RPTN, LGI1, CA11, NGFR, EGFL7, SERPINB2, SPAG6, HBA2, RTN4RL2, IGF1, LYZ, GDF6, MMP9, APLN, MFAP5, CHIT1, KISS1, MMP13, NRN1L, OAS1, PAPP, ADM2, SAA1, MMP19, CNTN3.</i>

	GO:0005615; extracellular space	43	1.61E-05	<i>DEFB103B, CPM, COL15A1, SERPINB12, CPB1, CPXM2, SERPINE1, CXCL13, GLDN, CXCL5, SERPINA5, SCUBE2, RELN, EFEMP1, CHAD, NTF3, BRICD5, IL32, LGI1, LINGO4, EGFL7, SERPINB2, CMTM5, SSC5D, HBA2, RTN4RL2, IGF1, SERPINB7, C1QL4, LYZ, GDF6, MMP9, DLK1, APLN, CHIT1, KISS1, MMP13, KRT18, NRN1L, PAPP, MMP19, LCP1, INA.</i>
	GO:0005887; integral component of plasma membrane	30	0.001	<i>CHRM2, CSF3R, GAL3ST1, ABCB4, ADRB2, SLC5A1, SLC6A1, MCHR1, GRM1, GJA5, NPBWR1, GALR1, SLCO2B1, TRPM8, SLC38A4, NGFR, SLC14A1, SCARA5, SLC6A17, ADRA2A, MAS1, ADGRD1, SLC6A9, ADGRB3, SLCO4A1, P2RX1, CDHR1, ADAM8, PCDHB3, SCN2A.</i>
	GO:0009986; cell surface	16	0.0052	<i>NGFR, CPM, CNTNAP2, EGFL7, ADGRV1, RTN4RL2, PSG11, HSPA2, SLC6A1, GLDN, SCUBE2, MAS1, SLC9A3, KCNQ1, ADAM8, CD24.</i>
Molecular Function	GO:0005509; calcium ion binding	25	7.63E-06	<i>RASGRP2, CACNA1E, NCALD, NKD1, CDH8, RYR3, SCUBE2, CAPN9, EFEMP1, CDH22, MYL10, PAMR1, RPTN, EGFL7, ADGRV1, ACTN3, DLK1, VIL1, MMP13, CDHR1, ADAM8, PCDHB3, LCP1, PLCB2, PVALB</i>
Biological Process	GO:0007165; signal transduction	23	0.032	<i>IL32, GUCY1A2, NDRG4, COL15A1, CSF3R, CMTM5, DIRAS2, MX1, IGF1, RASGRP2, ARHGAP36, PLCXD2, CXCL5, GLDN, APLN, SCUBE2, FAM83E, RASSF2, PPP1R1A, DPYSL5, P2RX1, NTF3, GNAT1.</i>
	GO:0007155; cell adhesion	14	0.0115	<i>IL32, CNTNAP2, COL15A1, EGFL7, CSF3R, CDH8, RELN, PDZD2, CDHR1, CNTN3, PCDHB3, CD24, FREM2, ITGA9.</i>

Supplementary Table 6. Differentially expressed genes in Ground old, sedentary vs Ground young, athletic derived myobundles

Gene	Description	Fold Change	FDR
Upregulated Genes (31)			
ADH1B	All-trans-retinol dehydrogenase [NAD (+)]	5.50663	0.010542
AC093525.2	AC093525.2 readthrough	5.459093	0.009363
GRIA4	Glutamate receptor 4	5.317638	0.000103
SORCS1	VPS10 domain-containing receptor SorCS1	4.751788	5.84E-09
PGA4	Pepsin A-4	4.188893	5.66E-13
PGA3	Pepsin A-3	3.980967	8.90E-05
CD163	Scavenger receptor cysteine-rich type 1 protein M130	3.590494	0.001014
PGA5	Pepsin A-5	3.278504	1.29E-07
PCDHA10	Protocadherin alpha-10	3.194691	0.000562
CNTNAP3	Contactin-associated protein-like 3	2.952285	0.005689
PNLIPRP3	Pancreatic lipase-related protein 3	2.752608	0.002605
RASSF2	Ras association domain-containing protein 2	2.583977	0.001096
SEMA3E	Semaphorin-3E	2.503173	5.48E-05
EDIL3	EGF-like repeat and discoidin I-like domain-containing protein 3	2.478723	1.10E-19
SCUBE3	Signal peptide, CUB and EGF-like domain-containing protein 3	2.462808	0.040758
IFI44L	Interferon-induced protein 44-like	2.35222	0.000728
KCNJ4	Inward rectifier potassium channel 4	2.348139	0.002507
DOK6	Docking protein 6	2.323972	5.45E-05
GPRIN3	G protein-regulated inducer of neurite outgrowth 3	2.31117	0.013039
SNCA	Alpha-synuclein	2.306055	0.041873
BCHE	Cholinesterase	2.300941	0.01719
SCAMP5	Secretory carrier-associated membrane protein 5	2.270838	3.94E-13
CADM3	Cell adhesion molecule 3	2.23186	0.000649
MYMX	Protein myomixer	2.131104	0.002002
FAIM2	Protein lifeguard 2	2.122362	0.002104

GRIK2	Glutamate receptor ionotropic	2.122041	1.94E-15
ADORA2B	Adenosine receptor A2b	2.121165	2.30E-10
FAM155A	Transmembrane protein FAM155A	2.095744	0.000611
EPB41L4A	Band 4.1-like protein 4A	2.050197	0.033361
CPS1	Carbamoyl-phosphate synthase [ammonia], mitochondria	2.029799	4.82E-08
MAGEL2	MAGE-like protein 2	2.013006	0.039268
Downregulated Genes (46)			
AC048338.1	AC048338.1 readthrough	-9.17064	0.000221
BARX1	Homeobox protein BarH-like 1	-7.57498	3.17E-06
CISH	Cytokine-inducible SH2-containing protein	-4.93738	0.001598
KIAA0040	Uncharacterized protein KIAA0040	-4.70838	2.70E-10
GAL3ST1	Galactosyl ceramide sulfotransferase	-4.4283	0.002806
SOCS3	Suppressor of cytokine signaling 3	-4.36672	0.000293
ST20-MTHFS	ST20-MTHFS readthrough	-4.29621	0.001366
STEAP4	Metalloreductase STEAP4	-4.17598	0.009271
TAC1	Protachykinin-1	-3.57829	8.90E-05
ABCC12	ATP-binding cassette sub-family C member 12	-3.42956	0.029524
EDN2	Endothelin-2	-3.39887	0.003263
CH25H	Cholesterol 25-hydroxylase	-3.38424	0.007555
GALNT6	Polypeptide N-acetylgalactosaminyltransferase 6	-3.34097	3.33E-05
SMTNL1	Smoothelin-like protein 1	-3.32285	2.64E-05
IL13RA2	Interleukin-13 receptor subunit alpha-2	-3.01697	0.001616
NOS2	Nitric oxide synthase, inducible	-2.93535	0.034761
CHRNA9	Neuronal acetylcholine receptor subunit alpha-9	-2.87794	0.000649
ALDH1L1	Cytosolic 10-formyltetrahydrofolate dehydrogenase	-2.87196	0.018974
ANO1	Anoctamin-1	-2.866	0.005077
RRAD	GTP-binding protein RAD	-2.82683	0.002793
RORC	Nuclear receptor ROR-gamma	-2.6904	1.46E-16
PDE4B	cAMP-specific 3',5'-cyclic phosphodiesterase 4B	-2.65437	0.000553
MB	Myoglobin	-2.64115	0.000845

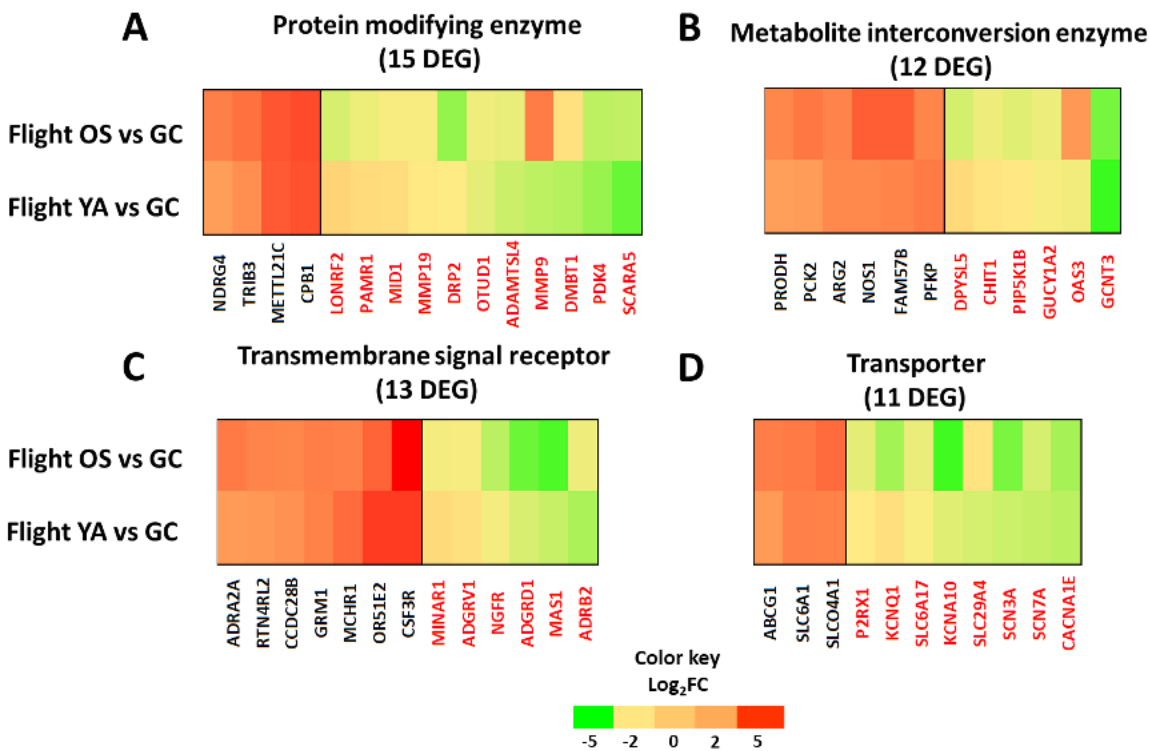
CALML4	Calmodulin-like protein 4	-2.59142	1.31E-06
SEC14L5	SEC14-like protein 5	-2.57624	0.001385
NNAT	Neuronatin	-2.49611	1.01E-20
NKX2-3	Homeobox protein Nkx-2.3	-2.47777	0.028421
RPS10-NUDT3	RPS10-NUDT3 readthrough	-2.43414	5.92E-13
KCNRG	Potassium channel regulatory protein	-2.42893	0.017351
FAM153A	Protein FAM153A	-2.41254	0.015294
NGEF	Ephexin-1	-2.39547	0.002088
SLC9A3	Sodium/hydrogen exchanger 3	-2.25413	0.049253
ZFP36	mRNA decay activator protein ZFP36	-2.25365	5.48E-07
RGS16	Regulator of G-protein signaling 16	-2.20432	0.03053
GABRE	Gamma-aminobutyric acid receptor subunit epsilon	-2.20095	0.000936
PLCB2	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-2	-2.19351	0.003512
CHL1	ATP-dependent DNA helicase DDX11	-2.16544	0.019322
TNXB	Tenascin-X	-2.15859	0.003185
SLX1A	Structure-Specific Endonuclease Subunit Homolog A,	-2.11698	0.003213
FOS	Proto-oncogene c-Fos	-2.11532	0.033171
CEBPD	CCAAT/enhancer-binding protein delta	-2.09462	9.32E-10
PLIN5	Perilipin-5	-2.0714	0.001877
GPR83	Probable G-protein coupled receptor 83	-2.0568	0.027382
CASQ1	Calsequestrin-1	-2.04761	0.000667
FOSB	FBJ murine osteosarcoma viral oncogene homolog B	-2.04146	0.010059
LLGL2	LLGL scribble cell polarity complex component 2	-2.00584	0.000611

Supplementary Table 7. Differentially expressed genes in Flight old, sedentary vs Flight young, athletic derived myobundles

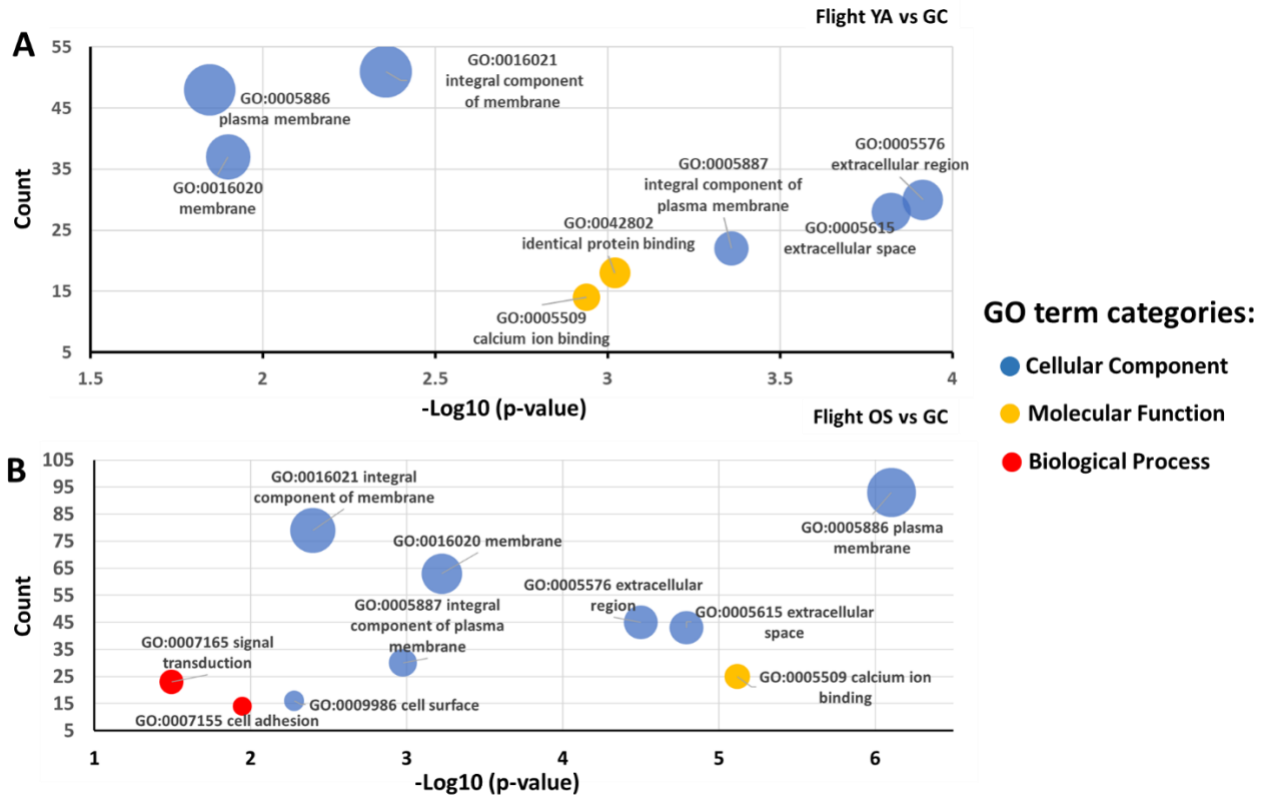
Gene	Description	Fold Change	FDR
Upregulated Genes (44)			
ADH1B	All-trans-retinol dehydrogenase [NAD(+)] ADH1B	6.476466606	0.004267711
SULT1A4	Sulfotransferase 1A4	6.46519923	0.009108532
PCDHA10	Protocadherin alpha-10	5.683026627	8.92125E-06
OAS1	2'-5'-oligoadenylate synthase 1	5.15144673	0.000673293
SORCS1	VPS10 domain-containing receptor SorCS1	4.481635518	1.21033E-11
CBSL	Cystathionine beta-synthase-like protein	4.441135365	0.018931424
MX1	Interferon-induced GTP-binding protein Mx1	4.149613978	1.13863E-07
CD163	Scavenger receptor cysteine-rich type 1 protein M130	3.737039114	0.002153255
MMP9	Matrix metalloproteinase-9	3.734812778	0.013499832
TMEM110-MUSTN1	TMEM110-MUSTN1 readthrough	3.706558203	0.000207655
COL28A1	Collagen alpha-1(XXVIII) chain	3.701382513	0.002712071
SYT1	Synaptotagmin-1	3.689683161	0.001941688
PGA3	Pepsin A-3	3.543055223	0.000678393
IFI44L	Interferon-induced protein 44-like	3.409816459	8.1528E-08
OAS3	2'-5'-oligoadenylate synthase 3	3.375568637	1.84192E-09
CADM3	Cell adhesion molecule 3	3.358524934	4.93045E-07
PGA4	Pepsin A-4	3.349734128	2.92566E-08
CNTNAP3	Contactin-associated protein-like 3	3.330953921	0.000212932
ANO2	Anoctamin-2	3.330086633	0.015099819
TFCP2L1	Transcription factor CP2-like protein 1	3.313716199	0.016620049
IFI27	Interferon alpha-inducible protein 27, mitochondrial	3.29935305	0.011949739
NOV	CCN family member 3	3.293005515	0.000473995
IFITM1	Interferon-induced transmembrane protein 1	3.222678754	1.84028E-05
EPSTI1	Epithelial-stromal interaction protein 1	3.148236733	0.008146499
SNCA	Alpha-synuclein	3.08909594	0.012642524
SFRP5	Secreted frizzled-related protein 5	3.008324388	0.012206691

SFRP1	Secreted frizzled-related protein 1	2.963010715	0.002429386
MYMX	Protein myomixer	2.841541397	9.32065E-06
EPHA3	Ephrin type-A receptor 3	2.770209347	0.002479832
GABRB3	Gamma-aminobutyric acid receptor subunit beta-3	2.739611434	0.000818999
PLA2G4A	Cytosolic phospholipase A2	2.641157626	0.005201275
EIF2S3B	Eukaryotic translation initiation factor 2 subunit 3B	2.465893665	0.000828289
FAM9C	Family with sequence similarity 9 member C	2.436032937	0.001740291
RIMS3	Regulating synaptic membrane exocytosis protein 3	2.432061242	0.001804696
ADORA2B	Adenosine receptor A2b	2.398180367	4.22786E-11
PWP2	Periodic tryptophan protein 2 homolog	2.36903012	0.000155964
TP53TG3D		2.345348958	0.042210595
PGA5	Pepsin A-5	2.330090118	0.000579969
FHAD1	Forkhead-associated domain-containing protein 1	2.311771577	0.031987124
EDIL3	EGF-like repeat and discoidin I-like domain-containing protein 3	2.305432437	1.21792E-15
SLC13A4	Solute carrier family 13 member 4	2.045267982	0.027502872
IFIH1	Interferon-induced helicase C domain-containing protein 1	2.028135888	0.000847821
SIK1	Serine/threonine-protein kinase SIK1	2.018609418	8.60471E-16
ACSS3	Acyl-CoA synthetase short-chain family member 3, mitochondrial	2.010982813	3.72392E-07
Downregulated Genes (22)			
NNAT	Neuronatin	-2.25377	8.6E-16
MAPK4	Mitogen-activated protein kinase 4	-2.38982	9.47E-10
MYH2	Myosin heavy chain 2	-2.14487	2.2E-08
STAC2	SH3 and cysteine-rich domain-containing protein 2	-2.13934	4.74E-08
OSTN	Osteocrin	-2.03364	8.09E-08
ACAN	Aggrecan core protein	-3.00922	1.55E-06
ZNF85	Zinc finger protein 85	-2.1116	5.89E-05
ARHGEF33	Rho guanine nucleotide exchange factor 33	-3.03639	6.74E-05
ACTN3	Alpha-actinin-3	-2.18816	0.000178
BARX1	Homeobox protein BarH-like 1	-6.23299	0.000242

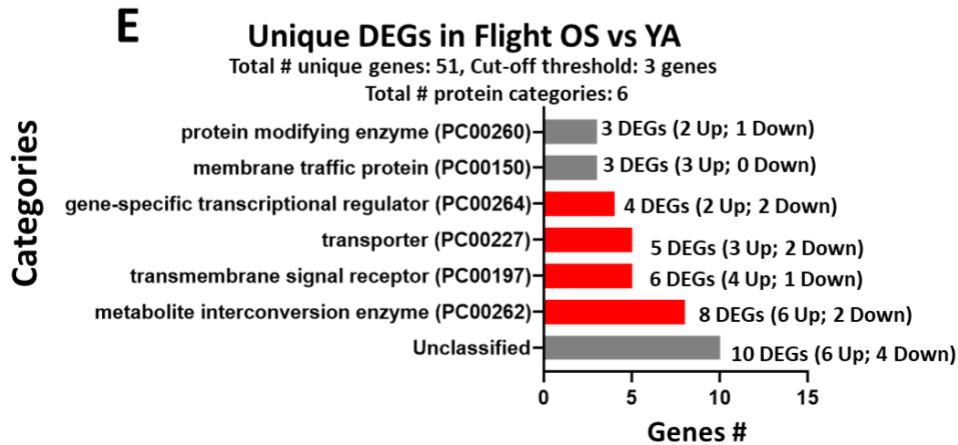
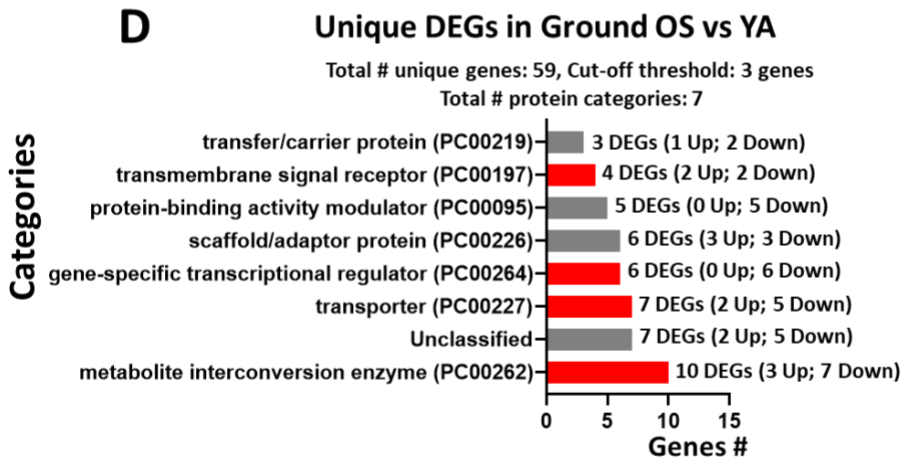
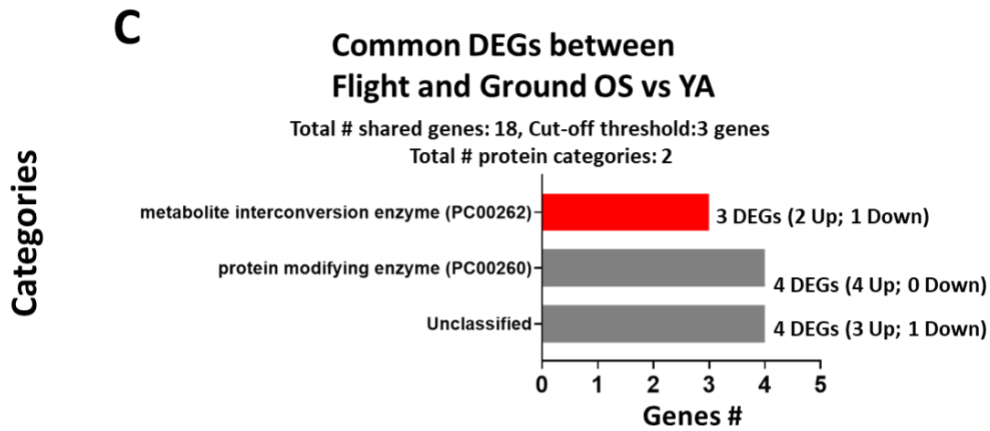
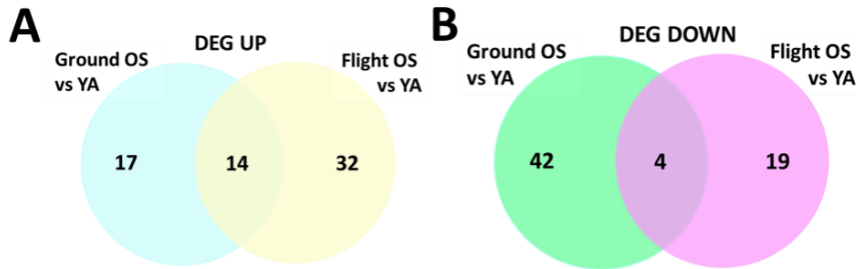
TSHZ2	Teashirt homolog 2	-2.25819	0.000374
PDZRN4	PDZ domain-containing RING finger protein 4	-2.34787	0.002614
WISP1	CCN family member 4	-2.91471	0.004592
TMEM154	Transmembrane protein 154	-2.33647	0.007467
ZNF781	Zinc finger protein 781	-3.92229	0.008469
ADGRB3	Adhesion G protein-coupled receptor B3	-4.23641	0.010137
COMP	Cartilage oligomeric matrix protein	-3.95436	0.012927
HIST1H1E	H1.4 linker histone	-3.91815	0.013631
TRPV3	Transient receptor potential cation channel subfamily V member 3	-2.29342	0.021744
GALNT6	Polypeptide N-acetylgalactosaminyltransferase 6	-2.05224	0.021867
B3GNT4	N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase 4	-2.43406	0.027832
ABCC3	ATP-binding cassette sub-family C member 3	-2.00106	0.041299



Supplementary Figure 1. Panther classification system analysis. Comparative heat map gene expression of the overlapping DEGs among the mean YA and mean OS flight versus ground datasets. DEGs associated with microgravity were normalized to GC and color coded as log₂FC from -5 to 5, with a p-value ≤ 0.05 . The log₂ fold change (log₂FC) values for the involved genes were extracted and displayed as heatmaps. Each chip is derived from cells pooled from 5 donors of the YA cohort or the OS cohort. Overrepresentation is shown as a significant fold enrichment based on the PANTHER overrepresentation test using Fisher's exact test with FDR multiple correction ($P \leq 0.05$) and *Homo Sapiens* as reference organism. Three chips from each cohort are used in the analysis.



Supplementary Figure 2. Gene ontology (GO) annotation. Bubble plots of differentially expressed gene sets overrepresented in the DAVID GO database. The analysis was performed separately for each experimental group. **A.** Flight YA vs. GC and **B.** Flight OS vs. GC. The graphs show only the GO terms above the established cut-off criteria (p -values ≤ 0.05 , $|\log_{2}FC| \geq 2$ and ≥ 14 genes per group). The x-axis represents the negative logarithm of the adjusted p value [false discovery rate (FDR)] for the GO terms, and the y-axis displays count, so each bubble's size reflects the number of differentially expressed genes, assigned to the GO terms. The top terms in each category, cellular component, molecular function, and biological process, are displayed in blue, orange, and red, respectively. The enriched functional GO terms associated with our gene list are identified and listed according to their enrichment P-value by DAVID using Fisher's Exact p-values test.



Supplementary Figure 3. Unique and common DEGs comparing ground OS vs YA and flight OS vs YA datasets. a. b. Venn diagrams showing the number of up-regulated and down-regulated DEGs identified by RNA-Seq among the two comparisons. DEGs only expressed in the ground comparison (light blue and green circles), only expressed in the flight comparison (yellow and circles), and common to both comparisons (intersections). c. d. e. Classification by Panther classification system of shared and unique DEGs shown in the Venn diagrams. The horizontal bar chart identifies the protein classes (unique gray, shared red) and relative number of DEGs of each functional class by applying a cut-off threshold of 3 genes. The protein categories are entitled with protein classifiers (PC) codes. Overrepresentation is shown as a significant fold enrichment based on the PANTHER overrepresentation test using Fisher's exact test with FDR multiple correction ($P \leq 0.05$) and *Homo Sapiens* as reference organism.