by Oct4 were differentiated *in vitro* into definitive endoderm (DE, yellow) marked by Sox17 and subsequently to pancreatic progenitors (PP, green), marked by the expression of Pdx1 and NKX6.1. The cells were transplanted into SCID-beige mice to complete maturation *in vivo*. (**K**, **L**) Immunostaining for Ucn3 (green) and insulin (red) on the *in vitro* differentiated cells shown at two magnifications (K, low magnification; L, high magnification). *In vivo* differentiated (transplanted) cells are shown in (**M**). Nuclei are stained with DAPI (blue). Scale bars = 50μm. Ucn3 is expressed in the *in vivo* matured cells, but not in *in vitro* differentiated insulin-positive β-like cells.

Supplementary Figure 1: Ucn3 expression in mouse islets is restricted to β-cells.

(A-C) Confocal images showing immunostaining of Ucn3 (green) and glucagon (red) on adult mouse pancreatic sections. **(D-F)** Ucn3 (green) and somatostatin (red) **(G-I)** Ucn3 (green) and pancreatic polypeptide (PPY, red). Nuclei are stained with DAPI (blue). Scale bars = 50μm. No co-localization of Ucn3 is seen with any of the islet hormones (other than insulin – see Fig 2 and 3).

Supplementary Figure 2: Ucn3 expression levels increase gradually in all β-cells during maturation, whereas insulin content stays constant.

Intra-cellular FACS analysis of insulin and Ucn3 in E18.5 (blue), P6 (green) and P13 (red). Histograms present the signal intensity of Ucn3 (A) and insulin (B) plotted against the percentage of all insulin expressing cells. Numbers in brackets show mean intensity ± sem of three independent biological repeats (three separate litters) for each age group.

Supplementary Figure 3: Ucn3 expression in human pancreas

(A-C) Confocal images showing immunostaining of Ucn3 (green) and insulin (red) on pancreatic sections from an adult human. **(D-F)** Ucn3 (green) and glucagon (red)**. (G-I)** Ucn3 (green) and somatostatin (red) **(J-L)** Ucn3 (green) and pancreatic polypeptide (PPY, red). Nuclei are stained with DAPI (blue). Scale bars = 50μm.

Supplementary Figure 4: HESC-derived β-cells secrete human C-peptide in response to glucose challenge

Mice transplanted with 5 million HESC-derived pancreatic progenitors were fasted 12 weeks after transplantation over night and injected with 2mg/kg glucose. The levels of human C-peptide before (fasting, blue) and one hour after glucose administration (glucose, red) are shown. Despite variable basal levels of human C-peptide, all animals except mouse #4, showed glucose-stimulated secretion of human C-peptide.

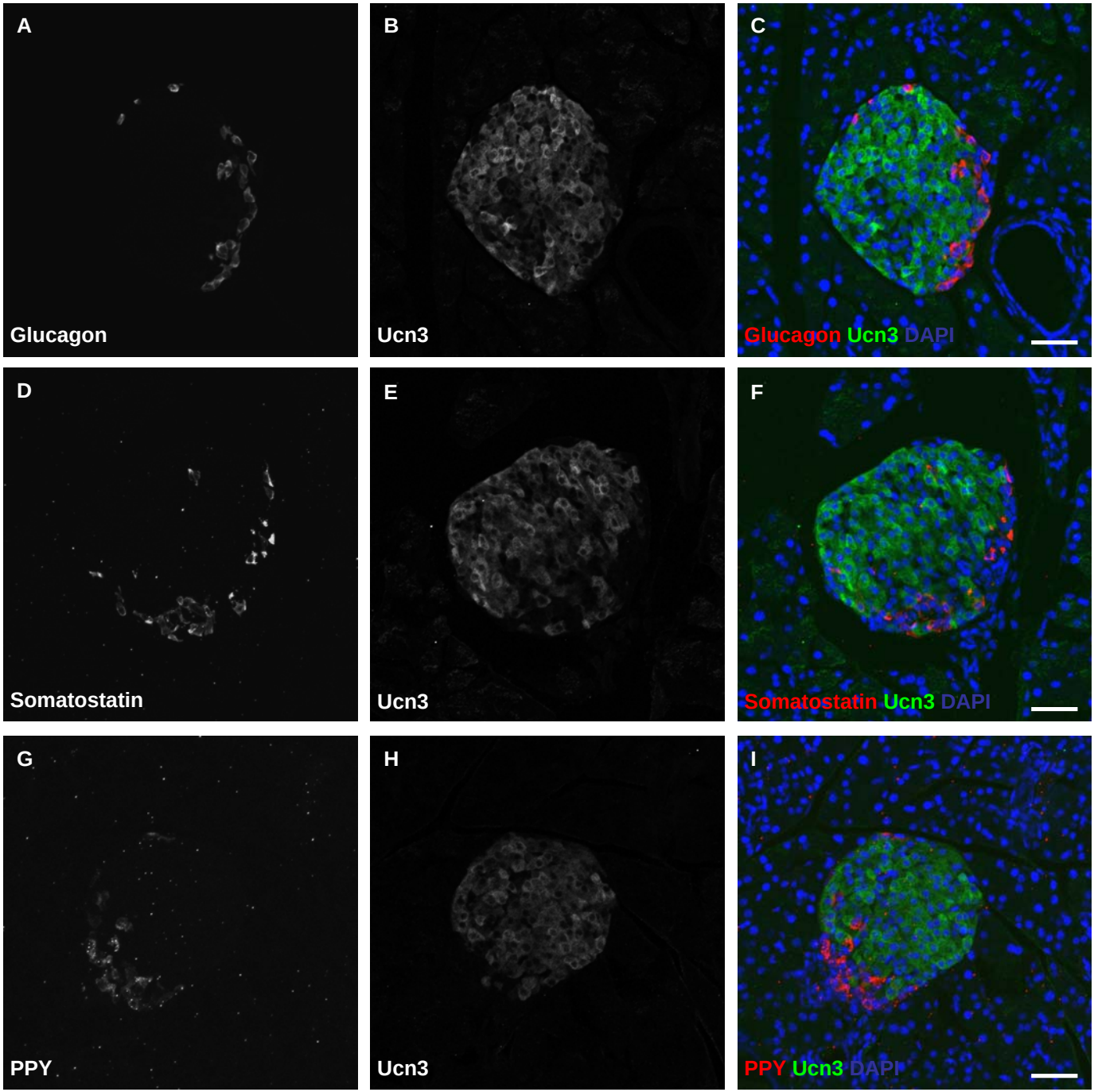
Supplementary Figure 5: Ucn3 expression in HESC-derived β-cells after maturation *in vivo*

(A-C) Confocal images showing immunostaining of Ucn3 (green) and insulin (red) on HESC-derived graft 8 months post transplantation. **(D-F)** Ucn3 (green) and glucagon (red)**. (G-I)** Ucn3 (green) and somatostatin (red) **(J-L)** Ucn3 (green) and pancreatic polypeptide (PPY, red). Nuclei are stained with DAPI (blue). Scale bars = 50μm.

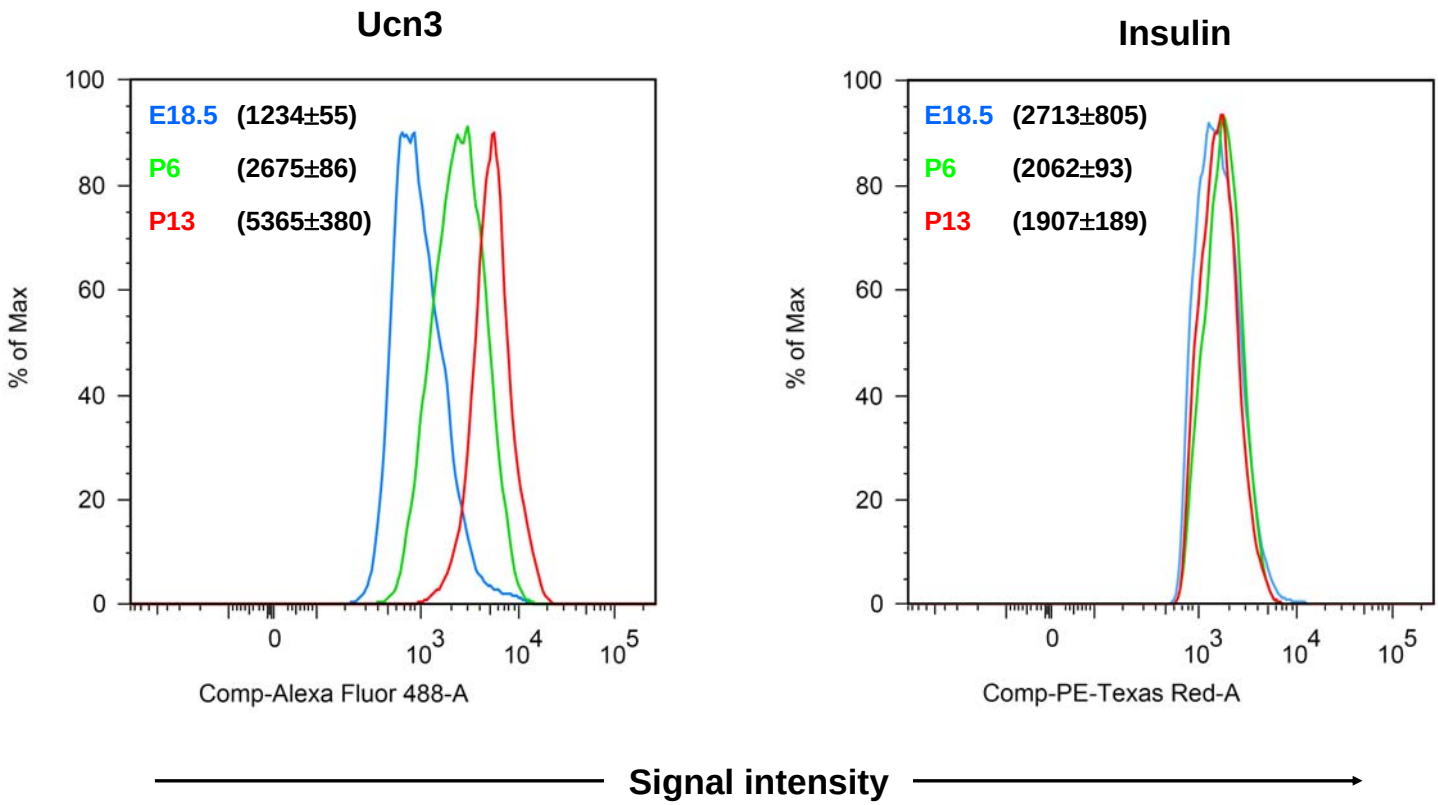
Supplementary Table 1

List of genes differentially expressed between immature (E18.5 and P1) and mature (P10 and adult) β-cells. Numbers represent normalized expression levels in the microarray ± standard deviation. Asterisks mark acinar-related genes. Underlined genes were chosen for analysis at the protein levels.

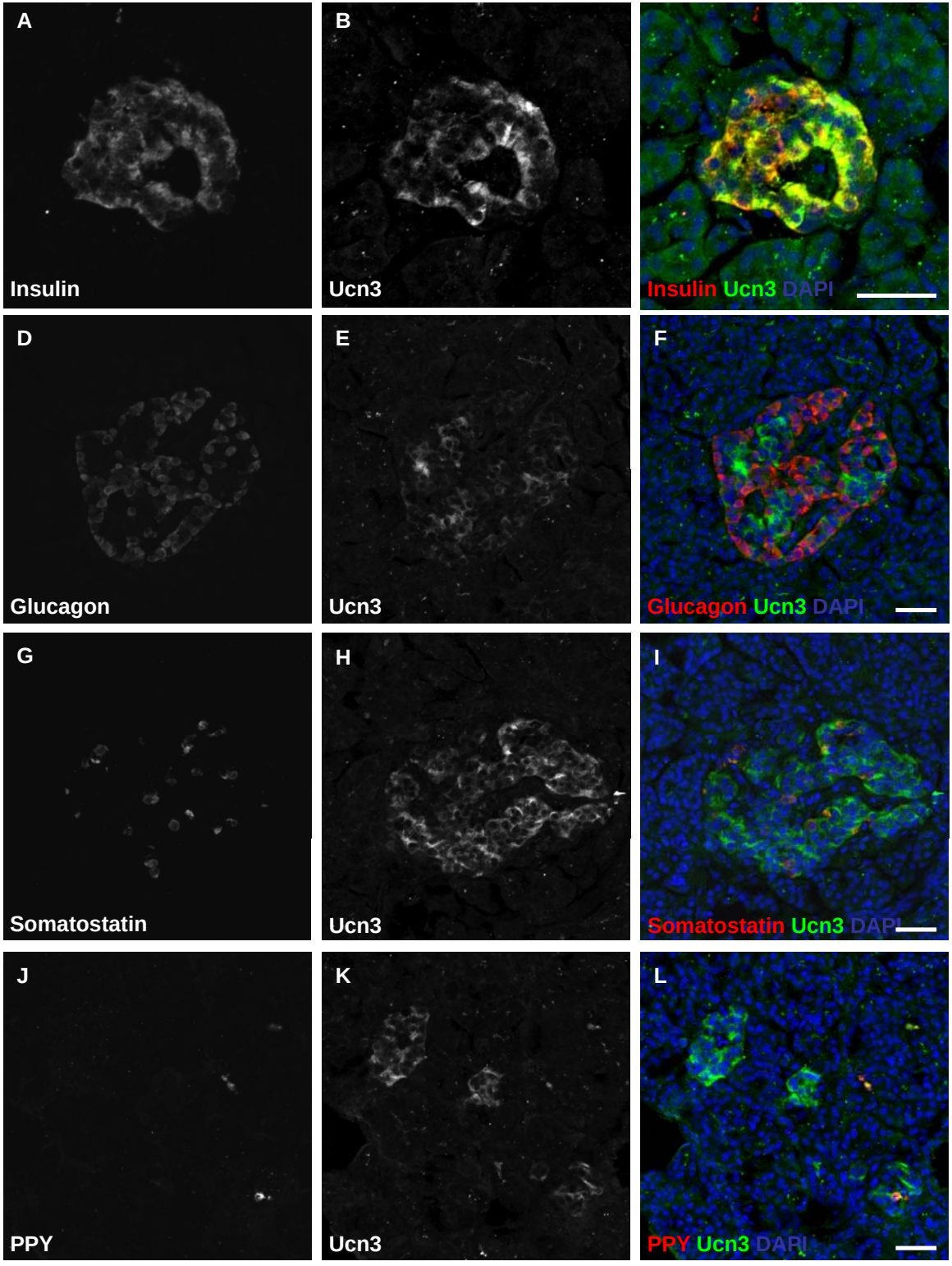
Supplementary Figure 1



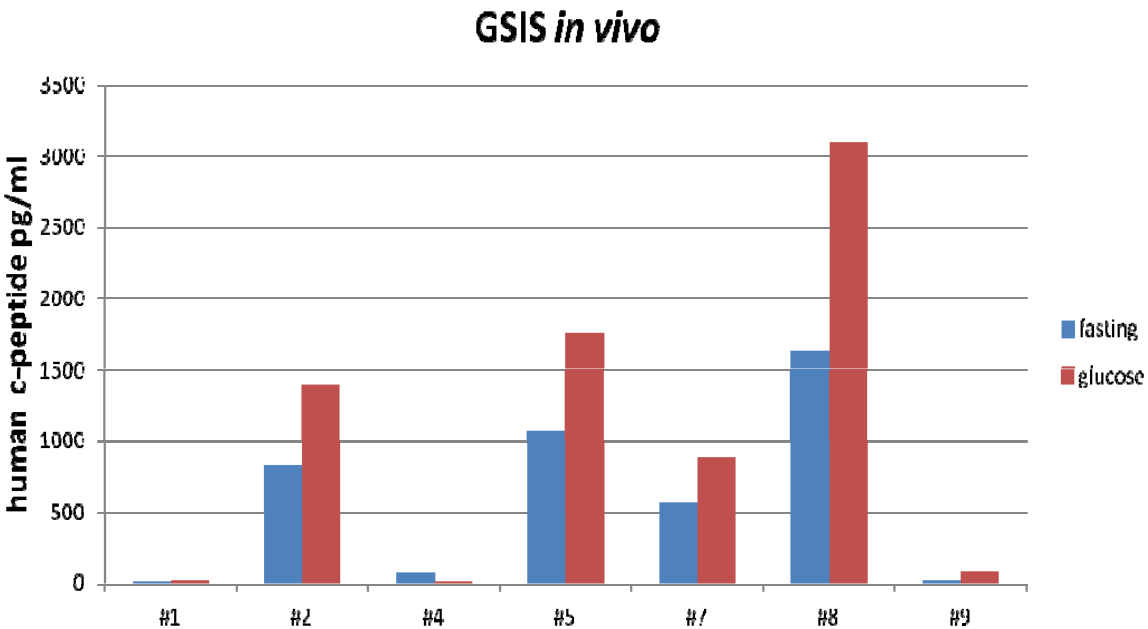
Supplementary Figure 2



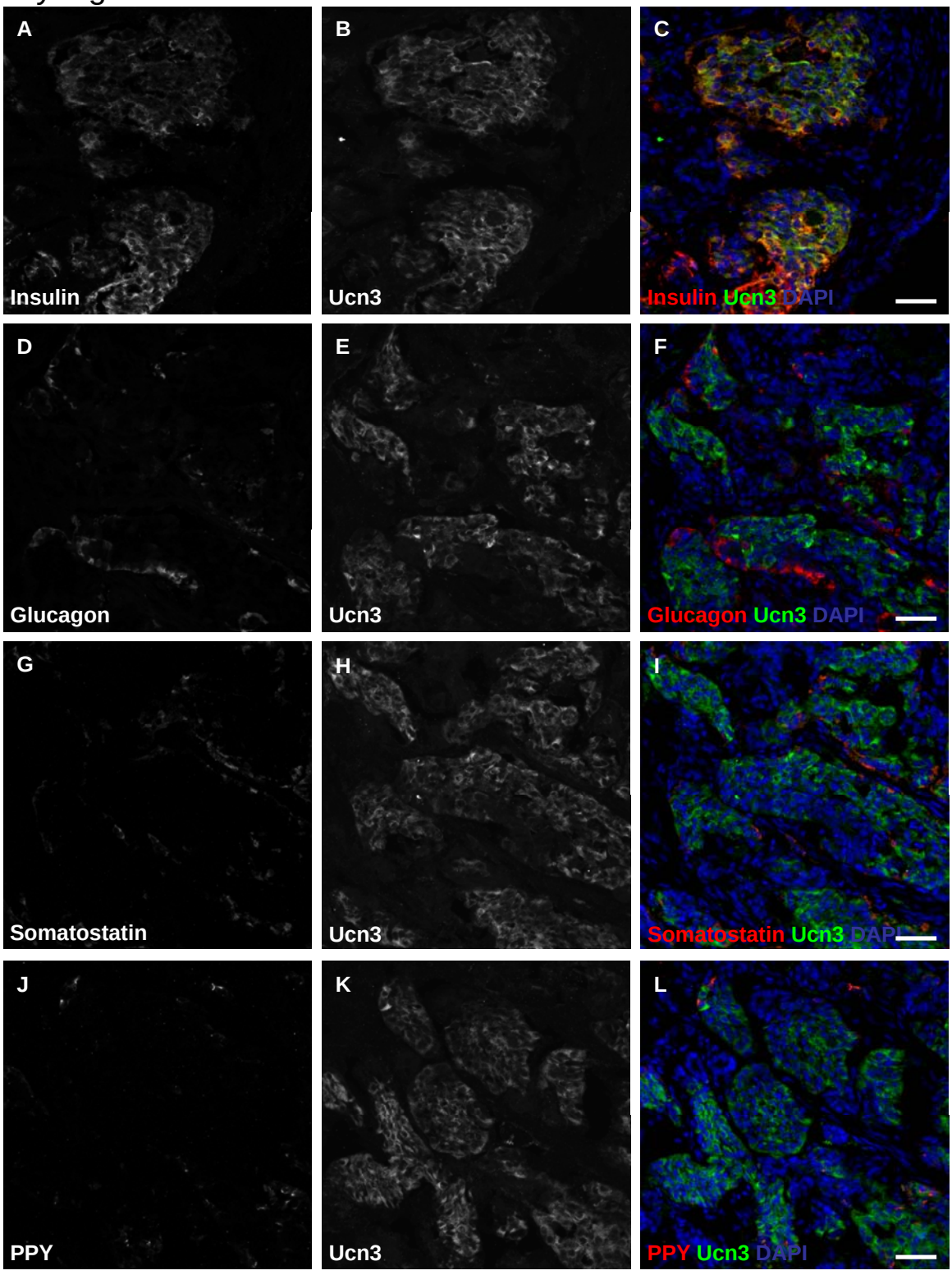
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary table 1

Up regulated genes

PROBE_ID	Gene symbol	E18.5	P1	P10	Adult	Fold change
ILMN_2673260	*Ctrl**	49 ± 8	69 ± 30	2269 ± 2057	3110 ± 1910	59.1
ILMN_2881083	*Try10**	65 ± 29	300 ± 312	4572 ± 3844	7237 ± 4442	39.7
ILMN_2716989	*Prss2**	46 ± 6	81 ± 54	1663 ± 1192	2392 ± 1334	39.4
ILMN_3160208	*Cpb1**	48 ± 17	83 ± 51	1627 ± 1322	2169 ± 1747	34.7
ILMN_2693403	*Ela1**	88 ± 26	102 ± 33	1513 ± 758	5071 ± 2642	34.0
ILMN_2666677	*Cel**	60 ± 23	95 ± 48	2100 ± 1965	1871 ± 1213	33.4
ILMN_2493756	*Try4**	49 ± 14	156 ± 141	1985 ± 1574	3706 ± 2554	33.1
ILMN_2728429	*1810010M01Rik**	53 ± 14	90 ± 52	1051 ± 632	3239 ± 2226	31.5
ILMN_2592415	*Reg1**	106 ± 71	202 ± 141	4954 ± 3212	3272 ± 2018	30.1
ILMN_2671137	*Ela3b**	57 ± 27	125 ± 94	1752 ± 1573	2280 ± 1351	26.7
ILMN_1220763	*Rnase1**	69 ± 38	128 ± 90	1891 ± 1290	3065 ± 2175	26.7
ILMN_2670847	*Cpa1**	89 ± 38	107 ± 52	1718 ± 1287	2728 ± 1855	24.6
ILMN_3104915	*1810049H19Rik**	47 ± 12	141 ± 122	1493 ± 1201	1953 ± 1200	23.3
ILMN_2919377	*Ctrb1**	106 ± 59	225 ± 203	3360 ± 2265	2670 ± 2042	21.2
ILMN_2933478	*Amy2-2**	47 ± 9	83 ± 43	428 ± 228	2166 ± 1554	20.2
ILMN_1246265	*Clps**	233 ± 182	467 ± 419	6237 ± 3526	6551 ± 4551	18.5
ILMN_2963762	*Try10l**	50 ± 15	163 ± 155	1272 ± 1049	1887 ± 1138	18.5
ILMN_2874291	*Amy2**	81 ± 45	205 ± 186	1080 ± 588	4393 ± 2661	18.3
ILMN_1216509	*Ctrc**	40 ± 4	43 ± 8	274 ± 196	1084 ± 920	17.9
ILMN_2990661	*Pnliprp2**	38 ± 5	47 ± 13	502 ± 355	697 ± 568	17.3
ILMN_2722659	*Clps**	86 ± 43	149 ± 113	1829 ± 1128	1668 ± 1335	16.7
ILMN_2674620	*Ela2**	253 ± 286	627 ± 576	5169 ± 3159	10910 ± 5518	16.5
ILMN_1232533	*Sycn**	46 ± 12	72 ± 34	462 ± 292	1374 ± 1022	16.5
ILMN_2829699	*EG436523**	51 ± 24	167 ± 160	1127 ± 957	1320 ± 996	14.1
ILMN_2708477	*Spink3**	52 ± 13	98 ± 76	993 ± 641	529 ± 383	13.3
ILMN_2760199	*Klk6*	57 ± 13	74 ± 23	564 ± 384	829 ± 609	12.3
ILMN_2904435	*Gp2**	37 ± 1	41 ± 7	181 ± 80	622 ± 493	11.2
ILMN_1225909	*Pnliprp1**	451 ± 380	774 ± 664	8347 ± 1724	4806 ± 2403	9.3

Up regulated genes (cont.)

PROBE_ID	Gene symbol	E18.5	P1	P10	Adult	Fold change
ILMN_2844820	*Angptl7*	68 ± 4	105 ± 16	383 ± 125	1043 ± 151	8.8
ILMN_1216566	*LOC232680**	61 ± 9	64 ± 13	452 ± 198	447 ± 349	8.2
ILMN_2731191	*Klk5**	46 ± 4	49 ± 10	238 ± 157	343 ± 269	7.5
ILMN_2684115	*2210010C04Rik**	37 ± 2	48 ± 13	201 ± 147	297 ± 184	7.4
ILMN_1238143	***Ucn3***	127 ± 20	154 ± 13	923 ± 22	1234 ± 285	7.2
ILMN_2690014	*Syt4*	80 ± 4	62 ± 5	252 ± 22	734 ± 107	6.9
ILMN_2692167	*Pnliprp2**	40 ± 2	43 ± 7	176 ± 130	264 ± 205	6.7
ILMN_1226556	*2310032F03Rik**	44 ± 3	47 ± 4	172 ± 15	435 ± 207	6.6
ILMN_2860932	*Zbtb2**	91 ± 13	76 ± 9	348 ± 211	629 ± 600	6.6
ILMN_1259215	*Serpina10*	136 ± 10	97 ± 25	275 ± 18	1303 ± 13	6.6
ILMN_1252131	*Klk1b27*	44 ± 5	52 ± 13	193 ± 151	270 ± 216	6.1
ILMN_1228211	*Tff2*	95 ± 3	81 ± 17	409 ± 215	446 ± 334	6.0
ILMN_1238736	*Klk1b4*	50 ± 3	58 ± 15	211 ± 141	308 ± 225	5.9
ILMN_1231724	*Resp18*	577 ± 54	684 ± 113	2693 ± 221	4401 ± 528	5.6
ILMN_2850077	*Adh1*	159 ± 21	195 ± 18	654 ± 25	1232 ± 221	5.1
ILMN_2824971	*Gpr158**	149 ± 7	163 ± 25	617 ± 88	900 ± 134	5.0
ILMN_2592718	*Cuzd1**	39 ± 4	39 ± 2	104 ± 58	235 ± 167	4.9
ILMN_1258501	*Adh1*	141 ± 23	161 ± 7	555 ± 19	999 ± 242	4.8
ILMN_2871660	*Car15*	52 ± 4	55 ± 1	364 ± 35	120 ± 45	4.7
ILMN_2968692	*Cpa2*	41 ± 5	44 ± 3	164 ± 80	152 ± 94	4.4
ILMN_2960700	*Prf1*	277 ± 43	249 ± 43	821 ± 302	1281 ± 918	4.2
ILMN_2804685	*Defb1*	69 ± 6	133 ± 42	319 ± 19	536 ± 126	4.2
ILMN_2596522	*Mt1**	958 ± 521	1348 ± 109	2719 ± 1045	7849 ± 4383	4.1
ILMN_2822825	*Fbxo2*	90 ± 1	111 ± 24	314 ± 50	401 ± 27	3.8
ILMN_2695819	*Ddit4l*	49 ± 4	50 ± 4	168 ± 51	149 ± 46	3.6
ILMN_2728038	*Arhgap24*	139 ± 16	128 ± 28	348 ± 33	595 ± 215	3.4
ILMN_2648742	*Abcb4*	78 ± 20	90 ± 19	266 ± 14	362 ± 46	3.4
ILMN_2839027	*Tceal6*	95 ± 9	102 ± 21	238 ± 10	447 ± 41	3.4
ILMN_2994299	*Hgfac*	182 ± 43	243 ± 18	547 ± 46	965 ± 91	3.3

Up regulated genes (cont.)

PROBE_ID	Gene symbol	E18.5	P1	P10	Adult	Fold change
ILMN_2628647	*Ddc*	560 ± 97	553 ± 76	1408 ± 133	2434 ± 256	3.3
ILMN_3064283	*Pde4dip*	115 ± 3	93 ± 17	245 ± 7	433 ± 45	3.3
ILMN_2681232	*D12Ertd647e*	216 ± 19	218 ± 20	492 ± 31	931 ± 203	3.2
ILMN_1254335	*Rgpr*	50 ± 3	47 ± 4	112 ± 13	194 ± 27	3.2
ILMN_3108770	*Fbxl10*	117 ± 18	116 ± 19	309 ± 1	489 ± 51	3.2
ILMN_1250689	*Rgs9*	133 ± 27	107 ± 14	361 ± 22	456 ± 49	3.1
ILMN_2624854	*Gstm2*	146 ± 23	140 ± 28	316 ± 36	603 ± 61	3.1
ILMN_1251449	*Gstm2*	144 ± 21	126 ± 11	303 ± 16	578 ± 64	3.1
ILMN_2601471	*Ccnd1*	177 ± 64	196 ± 13	473 ± 92	763 ± 165	3.0
ILMN_2959272	*Rnu6*	669 ± 62	511 ± 128	1627 ± 739	1383 ± 630	3.0
ILMN_3162403	*St6galnac3*	70 ± 4	79 ± 13	175 ± 3	280 ± 14	3.0
ILMN_2647234	*Dio1*	62 ± 9	65 ± 7	135 ± 13	248 ± 98	2.9
ILMN_3125606	*D12Ertd647e*	215 ± 22	214 ± 2	449 ± 29	800 ± 78	2.8
ILMN_2856567	*Ppy*	1597 ± 704	2056 ± 957	5681 ± 1998	4387 ± 3417	2.8
ILMN_2966162	*Tmem56*	88 ± 8	74 ± 20	188 ± 17	266 ± 91	2.8
ILMN_1217118	*Enpp5*	280 ± 46	281 ± 19	587 ± 2	1071 ± 343	2.7
ILMN_1221503	*Ccnd1*	206 ± 81	207 ± 21	466 ± 97	788 ± 134	2.7
ILMN_2862470	*Gstm2*	81 ± 11	77 ± 12	163 ± 0	270 ± 21	2.6
ILMN_2646640	*1700019D03Rik*	84 ± 7	91 ± 18	185 ± 2	278 ± 53	2.6
ILMN_2615096	*Dpp4*	119 ± 20	106 ± 17	312 ± 0	306 ± 19	2.5
ILMN_2652757	*Elovl5*	501 ± 20	510 ± 89	1153 ± 120	1247 ± 103	2.4
ILMN_2722996	*Ptpns1*	407 ± 77	424 ± 89	883 ± 13	1240 ± 203	2.4
ILMN_2731769	*Plekhb2*	149 ± 20	161 ± 24	341 ± 35	365 ± 60	2.2
ILMN_2652414	*Ncald*	146 ± 13	141 ± 23	312 ± 12	337 ± 31	2.2

Down regulated genes

PROBE_ID	Gene symbol	E18.5	P1	P10	Adult	Fold change
ILMN_1244618	*Dlk1*	2028 ± 195	2051 ± 315	126 ± 18	39 ± 1	-23.3
ILMN_2946520	*Npy*	3464 ± 545	2001 ± 551	235 ± 25	77 ± 38	-17.8
ILMN_2643658	*Ghrl*	1199 ± 617	643 ± 205	77 ± 27	40 ± 4	-17.1
ILMN_2755578	*Nnat*	1677 ± 588	1286 ± 499	257 ± 79	69 ± 17	-8.8
ILMN_2649773	*Slc38a5*	7037 ± 586	5761 ± 660	1181 ± 68	421 ± 122	-8.0
ILMN_1232456	*Nnat*	593 ± 146	448 ± 102	98 ± 28	42 ± 2	-7.1
ILMN_2598022	*Ghrl*	337 ± 147	173 ± 38	50 ± 2	43 ± 2	-6.9
ILMN_2518412	*Grb10*	1459 ± 160	933 ± 133	246 ± 11	155 ± 50	-6.2
ILMN_2635700	*Lgi2*	823 ± 136	833 ± 113	131 ± 10	166 ± 63	-5.8
ILMN_1251414	*Nxf*	525 ± 159	510 ± 141	89 ± 27	94 ± 48	-5.7
ILMN_2643049	*Chst8*	419 ± 65	611 ± 109	113 ± 16	64 ± 7	-5.7
ILMN_2842601	*Gp9*	356 ± 64	240 ± 49	62 ± 15	48 ± 3	-5.3
ILMN_1215713	*Egr4*	514 ± 250	434 ± 157	130 ± 51	46 ± 4	-5.3
ILMN_2597769	*Igf2*	1412 ± 843	852 ± 433	302 ± 106	232 ± 92	-4.9
ILMN_2906728	*H19*	239 ± 186	122 ± 107	54 ± 18	41 ± 2	-4.8
ILMN_2629519	*Cryba2*	4385 ± 749	4145 ± 949	1153 ± 153	624 ± 50	-4.8
ILMN_2623983	*Egr2*	247 ± 110	186 ± 96	63 ± 10	45 ± 5	-4.6
ILMN_2597827	*Arc*	321 ± 147	288 ± 91	90 ± 29	46 ± 8	-4.6
ILMN_2619408	*Atf3*	198 ± 110	115 ± 55	47 ± 6	43 ± 4	-4.4
ILMN_2734712	*Ptpla*	551 ± 81	372 ± 48	139 ± 5	87 ± 13	-4.3
ILMN_2708203	*Cdkn1c*	262 ± 67	203 ± 34	67 ± 2	55 ± 3	-4.3
ILMN_1250438	*Mlp*	233 ± 41	190 ± 64	59 ± 7	47 ± 1	-4.1
ILMN_2592834	*Sct*	274 ± 35	177 ± 23	68 ± 3	49 ± 3	-4.0
ILMN_1218913	*Igf2bp3*	307 ± 30	254 ± 45	94 ± 15	39 ± 1	-4.0
ILMN_2834379	*Tgfbi*	175 ± 30	155 ± 8	47 ± 5	41 ± 4	-3.9
ILMN_2745551	*Olfml2b*	228 ± 23	133 ± 38	45 ± 6	48 ± 5	-3.8
ILMN_2687661	*Mfng*	385 ± 25	427 ± 76	157 ± 29	41 ± 9	-3.7
ILMN_2945030	*Col9a2*	258 ± 18	218 ± 30	82 ± 4	52 ± 2	-3.6

Down regulated genes (cont.)

PROBE_ID	Gene symbol	E18.5	P1	P10	Adult	Fold change
ILMN_2981542	*Mfap2*	189 ± 53	171 ± 50	59 ± 4	51 ± 8	-3.6
ILMN_2771237	*Lbp*	161 ± 119	251 ± 167	70 ± 4	74 ± 45	-3.6
ILMN_1226175	*Igf2bp3*	255 ± 16	187 ± 25	85 ± 4	39 ± 4	-3.6
ILMN_2718330	*Cish*	1136 ± 211	1235 ± 112	421 ± 78	226 ± 92	-3.6
ILMN_2747543	*Actn3*	192 ± 23	217 ± 37	66 ± 4	52 ± 4	-3.5
ILMN_1221750	*Lmyc1*	193 ± 18	216 ± 22	73 ± 9	39 ± 1	-3.5
ILMN_2900653	*Gadd45b*	396 ± 125	306 ± 38	105 ± 50	87 ± 18	-3.4
ILMN_2636403	*Axud1*	248 ± 155	169 ± 23	84 ± 11	75 ± 19	-3.4
ILMN_1244169	*Sftpd*	244 ± 32	258 ± 55	87 ± 6	66 ± 8	-3.4
ILMN_1222084	*Rem2*	564 ± 60	634 ± 197	229 ± 47	112 ± 47	-3.2
ILMN_1225764	*1700018O18Rik*	133 ± 18	150 ± 11	47 ± 4	43 ± 5	-3.2
ILMN_2828112	*Igfbpl1*	134 ± 12	106 ± 8	38 ± 2	38 ± 2	-3.2
ILMN_2776034	*Gal*	202 ± 18	185 ± 20	48 ± 5	75 ± 19	-3.2
ILMN_2445324	*Zfpm1*	426 ± 58	489 ± 80	205 ± 3	105 ± 22	-3.1
ILMN_2941790	*Cldn6*	433 ± 86	344 ± 39	148 ± 9	123 ± 45	-3.1
ILMN_2736478	*Doc2b*	187 ± 25	134 ± 52	63 ± 3	47 ± 2	-3.1
ILMN_2930602	*Doc2b*	176 ± 39	114 ± 54	57 ± 7	44 ± 4	-3.0
ILMN_1246201	*Cacna1h*	218 ± 44	141 ± 54	62 ± 3	68 ± 21	-3.0
ILMN_2514292	*Zyx*	355 ± 86	264 ± 64	108 ± 16	112 ± 20	-3.0
ILMN_2678714	*Idb4*	195 ± 28	195 ± 30	84 ± 14	43 ± 1	-3.0
ILMN_2638324	*Gnas*	105 ± 34	121 ± 13	45 ± 4	40 ± 6	-2.9
ILMN_2700608	*Stx1a*	310 ± 45	341 ± 16	138 ± 12	93 ± 15	-2.9
ILMN_2718217	*2310057H16Rik*	248 ± 58	195 ± 36	91 ± 3	80 ± 5	-2.9
ILMN_2715546	*Gpx3*	3096 ± 199	3125 ± 456	1018 ± 207	1034 ± 229	-2.8
ILMN_2731407	*Gdf3*	131 ± 23	108 ± 15	44 ± 1	48 ± 6	-2.8
ILMN_1240857	*Cox7a1*	149 ± 26	128 ± 28	58 ± 3	46 ± 5	-2.8
ILMN_2714031	*1300002F13Rik*	716 ± 184	576 ± 164	278 ± 3	246 ± 42	-2.8
ILMN_1256702	*S100a10*	574 ± 73	455 ± 94	157 ± 36	200 ± 72	-2.8
ILMN_2756704	*9130213B05Rik*	195 ± 32	161 ± 27	78 ± 1	61 ± 4	-2.8

Down regulated genes (cont.)

PROBE_ID	Gene symbol	E18.5	P1	P10	Adult	Fold change
ILMN_2728379	*Ivd*	852 ± 99	1010 ± 106	368 ± 11	325 ± 51	-2.8
ILMN_2604029	*Klf2*	252 ± 98	187 ± 63	89 ± 42	66 ± 16	-2.7
ILMN_2632206	*Gnas*	1494 ± 455	1575 ± 250	708 ± 41	609 ± 135	-2.6
ILMN_2731949	*Copeb*	273 ± 86	238 ± 81	108 ± 33	91 ± 33	-2.6
ILMN_2430220	*Tmem2*	174 ± 34	164 ± 16	77 ± 5	65 ± 6	-2.5
ILMN_1248740	*Sema3f*	138 ± 31	123 ± 25	60 ± 7	49 ± 6	-2.5
ILMN_2766651	*Mafb*	115 ± 11	104 ± 33	47 ± 3	41 ± 2	-2.5
ILMN_3008110	*Actn3*	136 ± 15	141 ± 22	64 ± 2	57 ± 5	-2.4
ILMN_2735184	*Col18a1*	259 ± 79	210 ± 61	92 ± 56	86 ± 6	-2.4
ILMN_2996683	*Pvrl2*	376 ± 21	320 ± 43	159 ± 16	134 ± 34	-2.3
ILMN_2697361	*B930096L08Rik*	121 ± 16	120 ± 21	60 ± 6	47 ± 5	-2.3
ILMN_2745614	*1810015C04Rik*	278 ± 37	248 ± 94	122 ± 4	122 ± 33	-2.3
ILMN_3133448	*Mfge8*	412 ± 138	409 ± 52	165 ± 87	195 ± 77	-2.1
ILMN_2646625	*Jun*	456 ± 106	460 ± 158	220 ± 74	196 ± 90	-2.1
ILMN_2696299	*D5Ertd579e*	509 ± 27	523 ± 128	245 ± 50	245 ± 51	-2.0