Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information for:

Metabolic suppression during mesodermal differentiation of embryonic stem cells identified by single-cell comprehensive gene expression analysis

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Tables

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Table S1. Primer set list (48 genes) for STA.

Gene	Left Primer	Right Primer	Amplicon(bp)
Gapdh	tgtccgtcgtggatctgac	cctgcttcaccaccttcttg	75
Actb	aaggccaaccgtgaaaagat	gtggtacgaccagaggcatac	110
Tbp	ccaatgactcctatgacccctа	cagccaagattcacggtagat	104
Nanog	ttcttgcttacaagggtctgc	agaggaagggcgaggaga	67
Pou5f1	tgggcgttctctttggaa	gttgtcggcttcctccac	128
Sox17	cacaacgcagagctaagcaa	cgcttctctgccaaggtc	65
Gata6	ggtctctacagcaagatgaatgg	tggcacaggacagtccaag	94
Sox7	cacgctgcctgagaaaaac	gggagtactcacccctgtcc	62
Nes	ctgcaggccactgaaaagtt	tctgactctgtagaccctgcttc	73
Syn1	cccagatggttcgactacaca	ccacagggtatgttgtgctg	111
Ncam1	cagctcactttgtgttcagga	acaatgaggatgcccacaat	106
T	actggtctagcctcggagtg	ccattgctcacagaccagag	112
Nkx2-5	gacgtagcctggtgtctcg	gtgtggaatccgtcgaaagt	70
Myh7	ctggagaaagagaaaagcgagt	aggtccggcacatcttctc	106
Myh6	cgcatcaaggagctcacc	cctgcagccgcattaagt	61
Gata4	cccctcattaagcctcagc	caccctcggcattacgac	109
Myl7	gaaggagacctattcccagctc	gagtgtgaggaagacggtgaa	109
Kdr	ccccaaattccattatgacaa	cggctctttcgcttactgtt	69
Pecam1	gctggtgctctatgcaagc	atggatgctgttgatggtga	61
Etv2	cattgactcgctactccaaaact	tggaactcgcggctattg	127
Flii	gtctgaaggcttgccaggta	cattacctttgcggaccag	90
Gata2	cactctggacacatcctaccc	tagcccatggcagtcacc	101
Runx1	ctccgtgctacccactcact	atgacggtgaccagagtgc	102
Klf1	ggcgaactttggcaccta	caggcataaggcttctctcc	130
Gata1	cgagaccatcgtcatttgtg	gggaacactggggttgaa	107
Sox6	gctgggtgatcctcgtgta	tcccttgaggtcaagtcctg	111
Aloda	tgccagtatgttactgagaaggtc	catgttgggcttcagcaa	96
Pfkl	ctggtgattggtggctttg	gctgggatgacacacatgac	101
Bpgm	gaaagtgaaacaaggcaaacaa	cacacagcctagacttagggaga	125
Hk2	aactccggatgggacagaa	cacacggaagttggttcctc	67
Hk1	gtggacgggacgctctac	ttcactgtttggtgcatgatt	62
Gpi1	tgaacaaggaggtgatgcag	gcccgattctcggtgtagt	112
Pgk1	aatctctgctgggcaagga	gaaagcggaggttttccag	117
Pgm2	tggtcggaacttctttaccag	aaaggagcggtccagcat	103
Eno1	gaggacactttcatcgcagac	ccagctcttcctcaattctga	124
Pklr	tttggcatcgaaagtggaa	gtcagcacccgcataatgtt	113
Ldha	ggcactgacgcagacaag	agcttgatcacctcgtaggc	77
Cs	ccatggaacataagccactatct	ttccgtgccagagcatatt	64
Pdha1	gtcacttgtgtgatggtcagg	gtggtccgtagggtttatgc	68
Idh3b	gcagaatatggcttctgagga	gctagttcacccttatactccattg	114
Ogdh	ctgaacgtgcttgcaaatgt	acatgcccaggtggtacttc	118
Dlat	agggaagggtgttcgttagtc	cttgatgattctgccttctgg	104
Dld	tgaaaacccctggttggag	gccatgagatatccgattgaa	106
Aco2	atctgattgaggcccaggtt	gaagcccacaccatacttgg	113
Idh2	caggtcaccattgactctgc	ccacattttcttcagcttgaact	114
Sdhb	tccattgagccttatctgaagaa	actcgtacagcccgtcca	103
Sdhc	cacctgaatgctcagctttgt	cggtttgaactcgtgttcttc	101
Fh1	gccatgccaagaatctacg	gcagtgacaaaaggcaaacc	131

Table S2. Primer set list (48 genes) for high-throughput single-cell qPCR.

Gene	Left Primer	Right Primer	Amplicon(bp)
Gapdh	CGTCGTGGATCTGACgtg	GCTTCACCACCTTCTTGatg	68
Actb	CCAACCGTGAAAAGATgacc	TACGACCAGAGGCATACagg	102
Tbp	AATGACTCCTATGACCCCTAtca	GCCAAGATTCACGGTAGATaca	100
Nanog	TGCTTACAAGGGTCTGCtactg	AGGAAGGGCGAGGAGAgg	61
Pou5f1	GAAaggtgttcagccagacc	CTTCCTCCACccacttctcc	105
Sox17	CAACGCAGAGCTAAGCAAgat	GCTTCTCTGCCAAGGTCaac	62
Gata6	AAGATGAATGGcctcagcag	gaaggcacgcgcttctgt	53
Sox7	GCCTGAGAAAAACggcatt	ACTCACCCCTGTCCtccttc	50
Nes	GCAGGCCACTGAAAAGTTcc	TAGACCCTGCTTCtcctgct	61
Syn1	CCAGATGGTTCGACTACACAag	CAGGGTATGTTGTGCTGctg	107
Ncam1	CTCACTTTGTGTTCAGGAcctc	ATGAGGATGCCCACAATgg	100
T	cagcccacctactggctcta	TTGCTCACAGACCAGAGactg	51
Nkx2-5	TAGCCTGGTGTCTCGgacct	GGAATCCGTCGAAAGTgc	62
Myh7	GGAGAAAGAGAAAAGCGAGTtc	CCGGCACATCTTCTCcag	100
Myh6	TCAAGGAGCTCACCtaccaga	GCAGCCGCATTAAGTtcttc	54
Gata4	cgggtaggcctctcctgt	cacagcgtggtggtggtagt	50
Myl7	AGCTCgggagggtaagtgtt	TGAGGAAGACGGTGAAgttg	87
Kdr	TGACAAcacagcaggaatcag	GCTCTTTCGCTTACTGTTctgg	52
Pecam1	TGCTCTATGCAAGCctccag	TGCTGTTGATGGTGAaggag	51
Etv2	CTaaccaccgaggtcccatt	gagcagctccaggaggaat	50
Flii	GGCTTGCCAGGTAtacatcc	gtggccgctcatgttcttta	53
Gata2	atccacccttcctccagtct	CCATGGCAGTCACCatgc	64
Runx1	ccatcgctttcaaggtggt	GGTGACCAGAGTGCcatcc	56
Klf1	CGAACTTTGGCACCTAagagg	TAAGGCTTCTCTCCcgtgtg	122
Gata1	GAGACCATCGTCATTTGTGtact	AACACTGGGGTTGAAccttg	103
Sox6	GGGTGATCCTCGTGTAtaactaa	GAGGTCAAGTCCTGggtcat	102
Aloda	ctgagcgaccaccatgtcta	ATGTTGGGCTTCAGCAAtgt	50
Pfkl	TTGaggcctacgagggtgt	TGGGATGACACACATGACaa	83
Bpgm	Ataaagtgtggccaggaacg	CAGCCTAGACTTAGGGAGAgg	100
Hk2	GGGACAGAAcatggcgagt	GGAAGTTGGTTCCTCcaagg	52
Hk1	GGGACGCTCTACaaactcca	TGTTTGGTGCATGATTctgg	51
Gpi1	AAGGAGGTGATGCAGatgct	CCCGATTCTCGGTGTAGTtg	106
Pgk1	CTGGGCAAGGAtgttctgtt	GGAGGTTTTCCAGcaggatg	103
Pgm2	ctacgaggaggtggaagctg	cctccaggtccttcatcatt	53
Eno1	CTTTCATCGCAGACctggtg	TTCTGAggatctgattgtacttgg	102
Pklr	TCGAAAGTGGAAagcttcgt	CACCCGCATAATGTTggtat	101
Ldha	GACAAGgagcagtggaagga	GTAGGCactgtccaccacct	51
Cs	GCCACTATCTtctgaccttgg	TCCGTGCCAGAGCATATTaac	50
Pdha1	TTGTGTGATGGTCAGGaagc	GGTTTATGCcagcctcca	52
Idh3b	GCTTCTGAGGAgaagctgga	tgatggcaactttgttctcct	61
Ogdh	GCTTGCAAATGTcatcagga	GCCCAGGTGGTACTTCatgt	106
Dlat	GGAAGGGTGTTCGTTAGTCct	TGATGATTCTGCCTTCTGGtc	100
Dld	atgcagagctggagtcgtgt	ATTGAAatggcctttcttgg	54
Aco2	gccaaggacataaaccagga	CACACCATACTTGGcacctg	63
Idh2	CACCATTGACTCTGCtctgg	CTTCAGCTTGAACTcttccaca	100
Sdhb	AAgaaggatgagtcccaggag	tcctcgatggactgcagata	53
Sdhc	cctttgggaaccacagctaa	TCGTGTTCTTCttccagaacc	55
Fh1	ATCTACGagctcgctgctg	AAGGCAAACCtgtgagtgct	109

Table S3. Comparison of average gene expression levels in the Flk-1(+) and Flk-1(-)

cells.

Gene	Category	pValue	Flk-1(-).Average	Flk-1(+).Average
Kdr	differentiation	9.7763E-06	9.1361	22.3843
Sox7	differentiation	3.6388E-05	1.3882	13.5679
Etv2	differentiation	3.2016E-04	11.1994	19.0212
Gata2	differentiation	9.9183E-04	5.8055	13.5876
Gata6	differentiation	1.7950E-03	10.3786	17.4651
Nanog	pluripotency	2.3762E-03	10.6921	5.2262
Pgm2	glycolysis	1.8231E-02	7.8085	13.0259
Pou5f1	pluripotency	3.4301E-02	14.8419	8.8900
Nkx2.5	differentiation	5.1649E-02	0.9720	5.1214
Syn1	differentiation	5.3251E-02	11.6608	5.9030
Pecam1	differentiation	6.2613E-02	3.7160	9.4708
Myl7	differentiation	8.0199E-02	1.4927	4.2555
Dlat	TCA cycle	1.0667E-01	19.6699	19.1688
Pdha1	TCA cycle	1.2883E-01	17.5063	17.0492
Runx1	differentiation	1.3587E-01	9.2169	13.8336
Tbp	house keeping	1.4104E-01	20.3815	19.9686
Gata4	differentiation	1.8005E-01	13.6595	16.7600
Sox17	differentiation	2.1314E-01	4.0939	7.6505
Idh2	TCA cycle	2.3568E-01	20.2019	19.8638
Gapdh	house keeping	2.3882E-01	25.1616	24.8261
T	differentiation	2.4023E-01	19.1949	17.2326
Pklr	glycolysis	2.4580E-01	0.7924	0.0000
Bpgm	glycolysis	3.2952E-01	16.4310	15.3003
Pfkl	glycolysis	3.8719E-01	15.9601	16.3463
Eno1	glycolysis	3.9304E-01	13.9403	13.6944
Klf1	differentiation	4.0459E-01	0.0000	0.6463
Sdhc	TCA cycle	4.9294E-01	19.9370	19.7778
Sdhb	TCA cycle	5.2820E-01	19.2768	19.1022
Hk2	glycolysis	5.5609E-01	18.4217	18.6406
Hk1	glycolysis	6.2053E-01	11.8482	13.0108
Cs	TCA cycle	6.4185E-01	21.0180	21.1310
Pgk1	glycolysis	6.4198E-01	20.9750	21.1125
Nes	differentiation	6.8384E-01	9.2825	10.1949
Myh6	differentiation	7.0255E-01	1.3734	0.7972
Ncam1	differentiation	7.3253E-01	7.7571	8.4811
Aco2	TCA cycle	8.3895E-01	20.2356	20.1841
Fh1	TCA cycle	8.5655E-01	20.6915	20.6369
Myh7	differentiation	8.6205E-01	1.7992	1.5573
Gpi1	glycolysis	8.9449E-01	18.8414	18.8804
Flii	differentiation	8.9774E-01	18.8549	18.8922
Ldha	glycolysis	9.0975E-01	22.7976	22.8314
Dld	TCA cycle	9.1617E-01	16.7173	16.6826
Sox6	differentiation	9.2270E-01	3.5162	3.7451
Ogdh	TCA cycle	9.3938E-01	19.9834	19.9595
Aloda	glycolysis	9.6432E-01	21.7229	21.7080

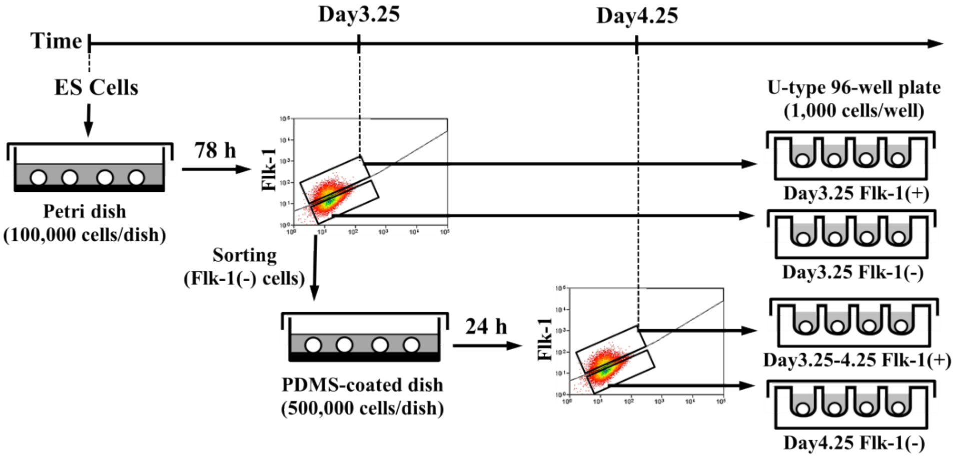

Fig. S1 Procedure of FACS and re-aggregation of four different cell groups.

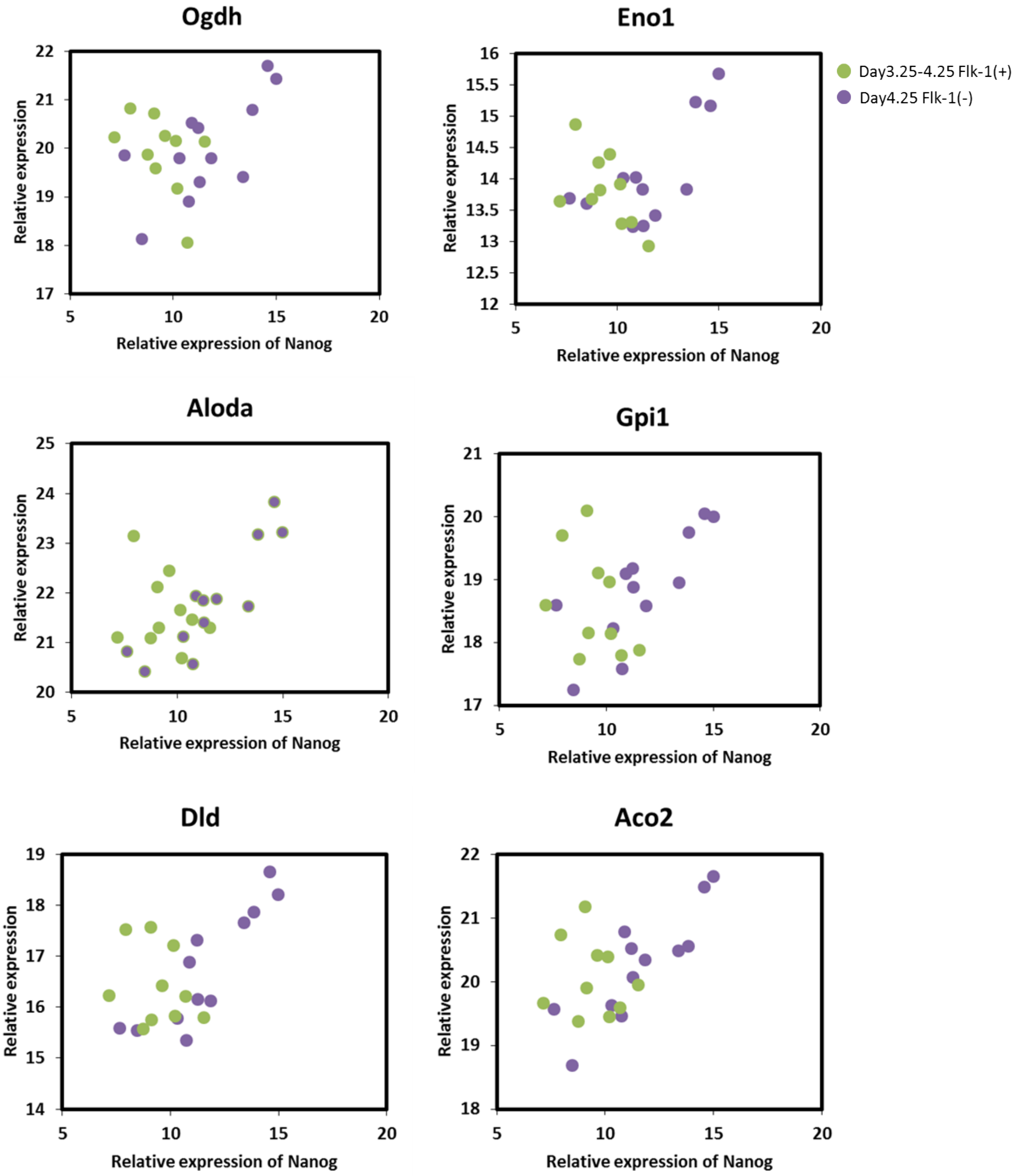

Fig. S2. The correlation of the top six correlated glucose metabolism-related genes (*Ogdh*, *Eno1*, *Aloda*, *Gpi1*, *Dld*, and *Aco2*, according to Table 2) as a function of the expression of *Nanog*.

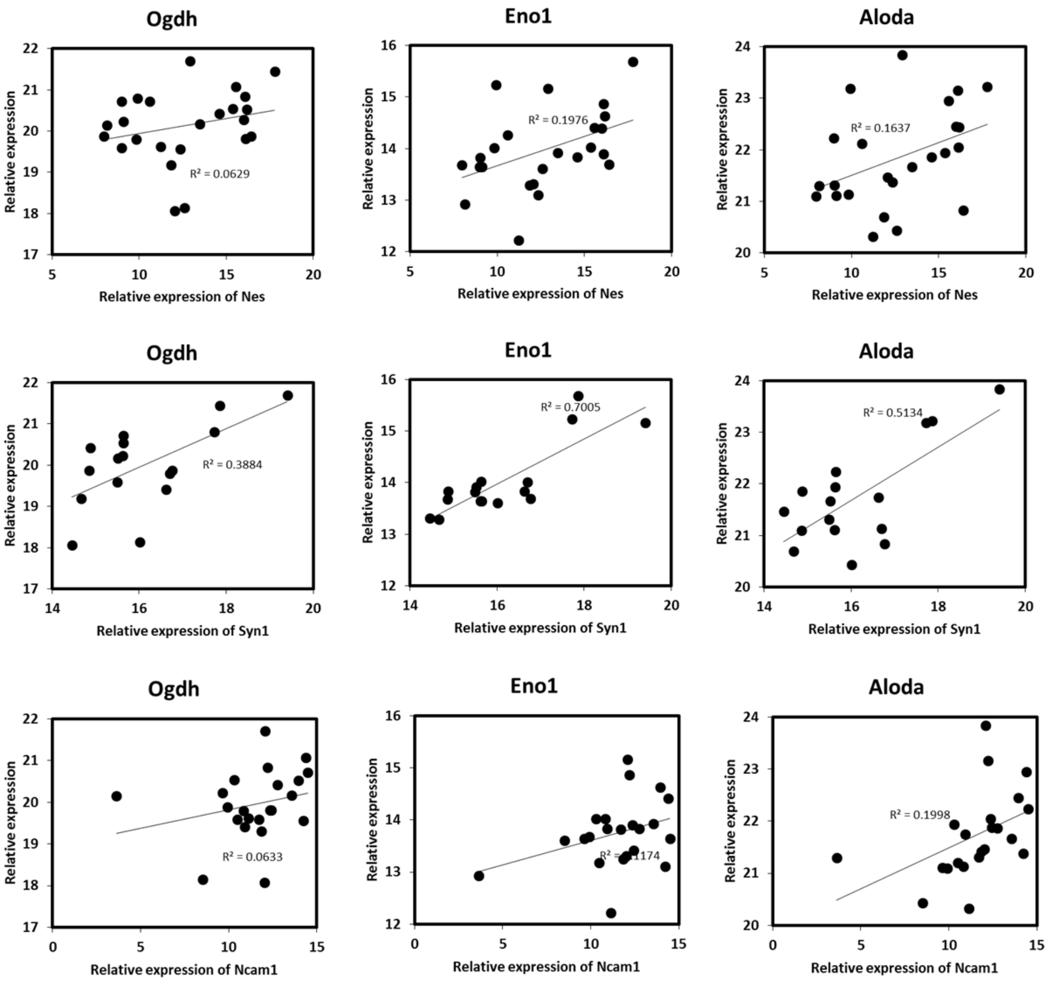

Fig. S3. The correlation of the top three correlated glucose metabolism-related genes (*Ogdh*, *Eno1*, *Aloda,* according to Table 2) as a function of the expression of ectoderm markers.

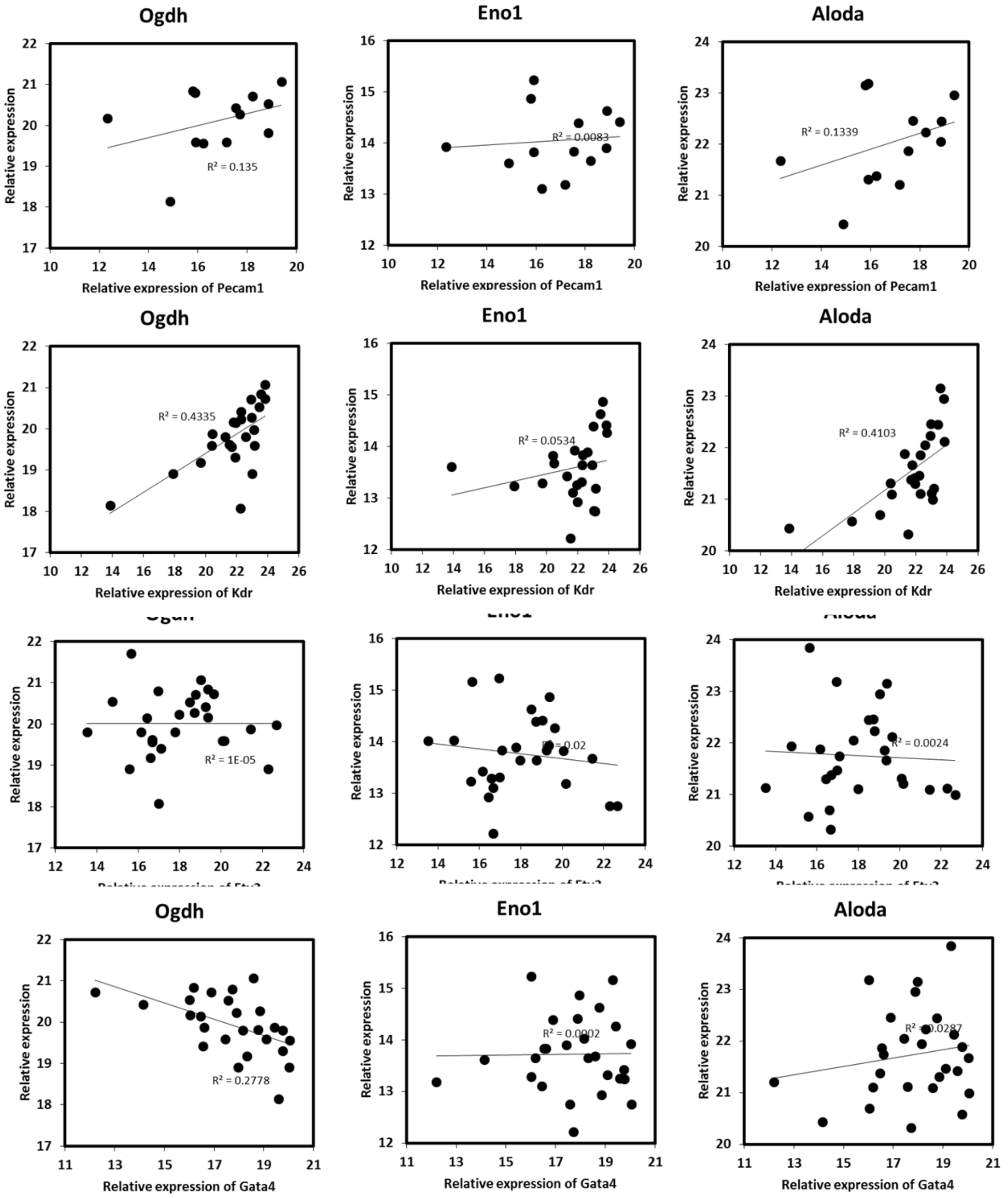

Fig. S4. The correlation of the top three correlated glucose metabolism-related genes (*Ogdh*, *Eno1*, *Aloda,* according to Table 2) as a function of the expression of mesoderm markers.

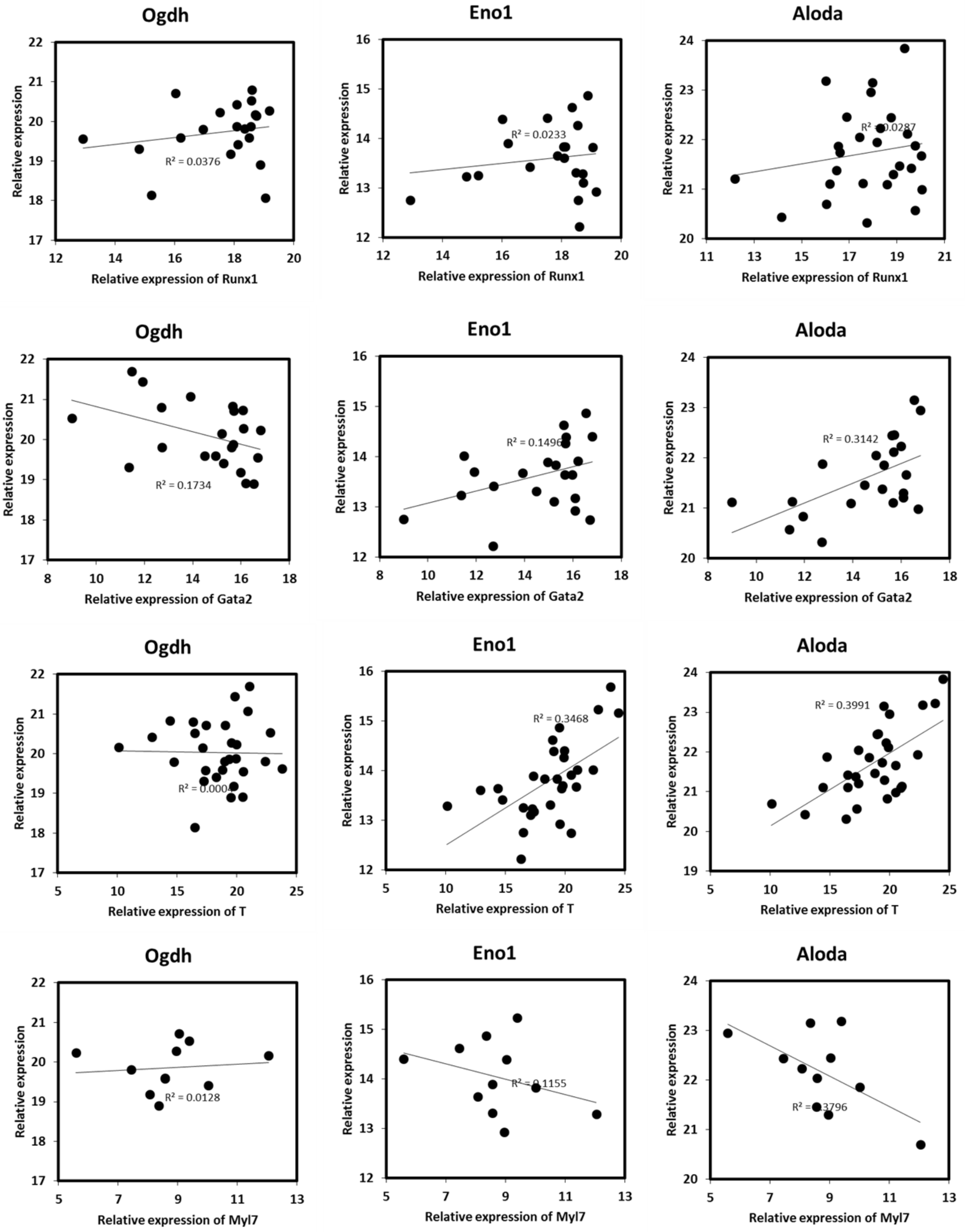

Fig. S4. The correlation of the top three correlated glucose metabolism-related genes (*Ogdh*, *Eno1*, *Aloda,* according to Table 2) as a function of the expression of mesoderm markers. (Continued)

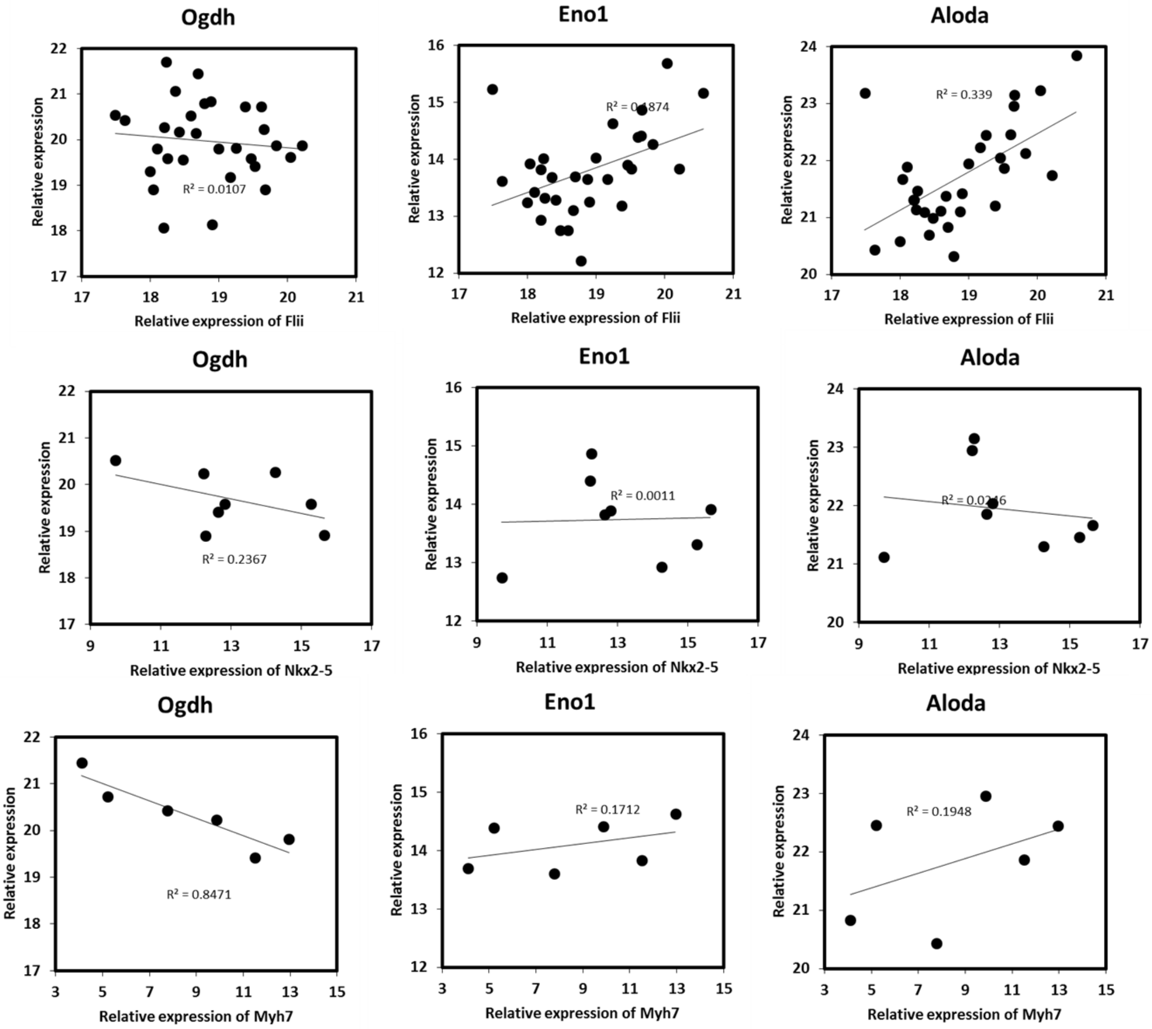

Fig. S4. The correlation of the top three correlated glucose metabolism-related genes (*Ogdh*, *Eno1*, *Aloda,* according to Table 2) as a function of the expression of <u>mesoderm</u> markers. (Continued)

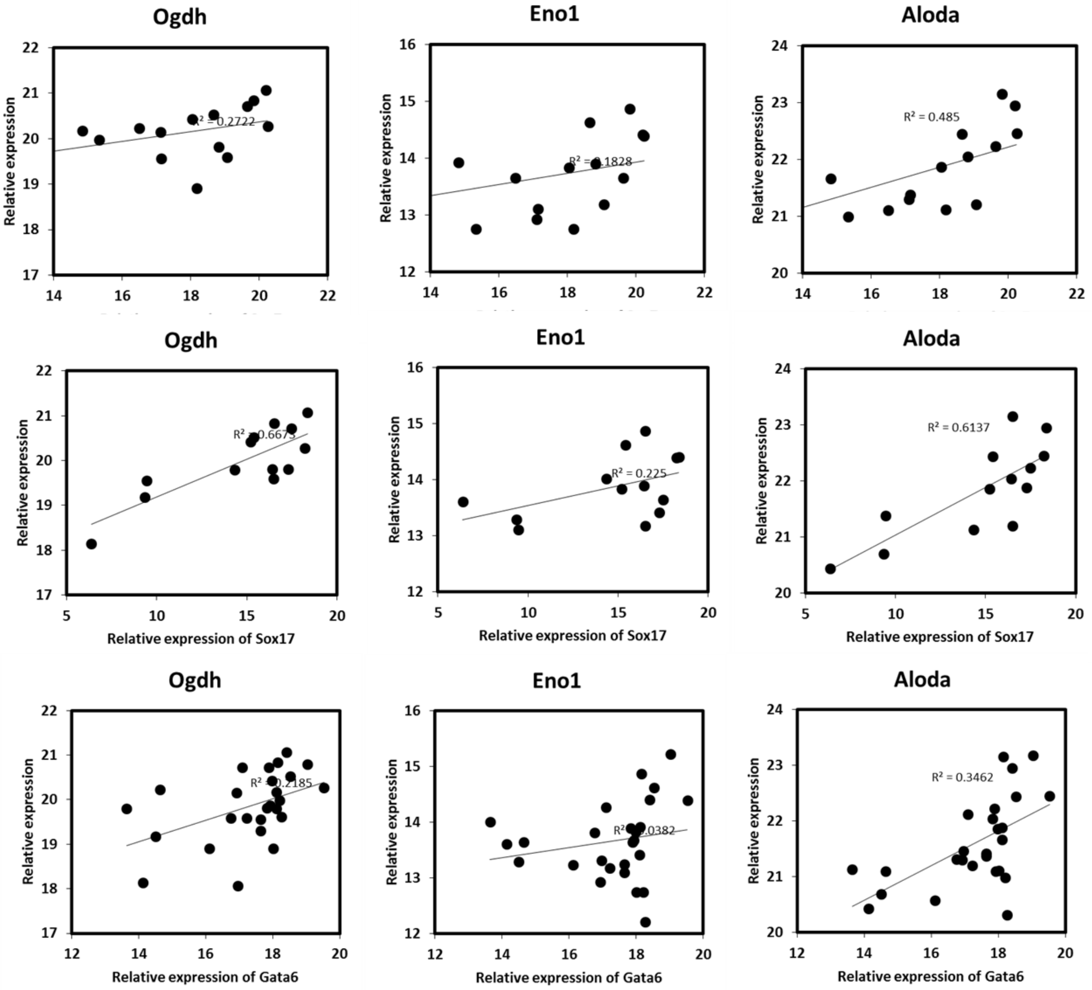

Fig. S5. The correlation of the top three correlated glucose metabolism-related genes (*Ogdh*, *Eno1*, *Aloda,* according to Table 2) as a function of the expression of endoderm markers.