Supporting Information

Durand et al. 10.1073/pnas.1201652109

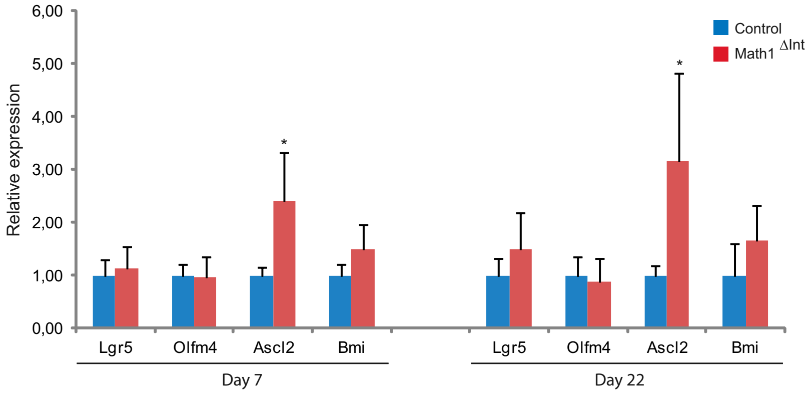


Fig. S1. Math1 expression is dispensable for stem cell marker expression. Real-time quantitative RT-PCR analysis of stem cell markers in the small intestine of control and Math1$^{\Delta int}$ mice 7 and 22 d after Tam injections. No significant differences were observed except for those marked by an asterisk, where $^{*}P < 0.05$.

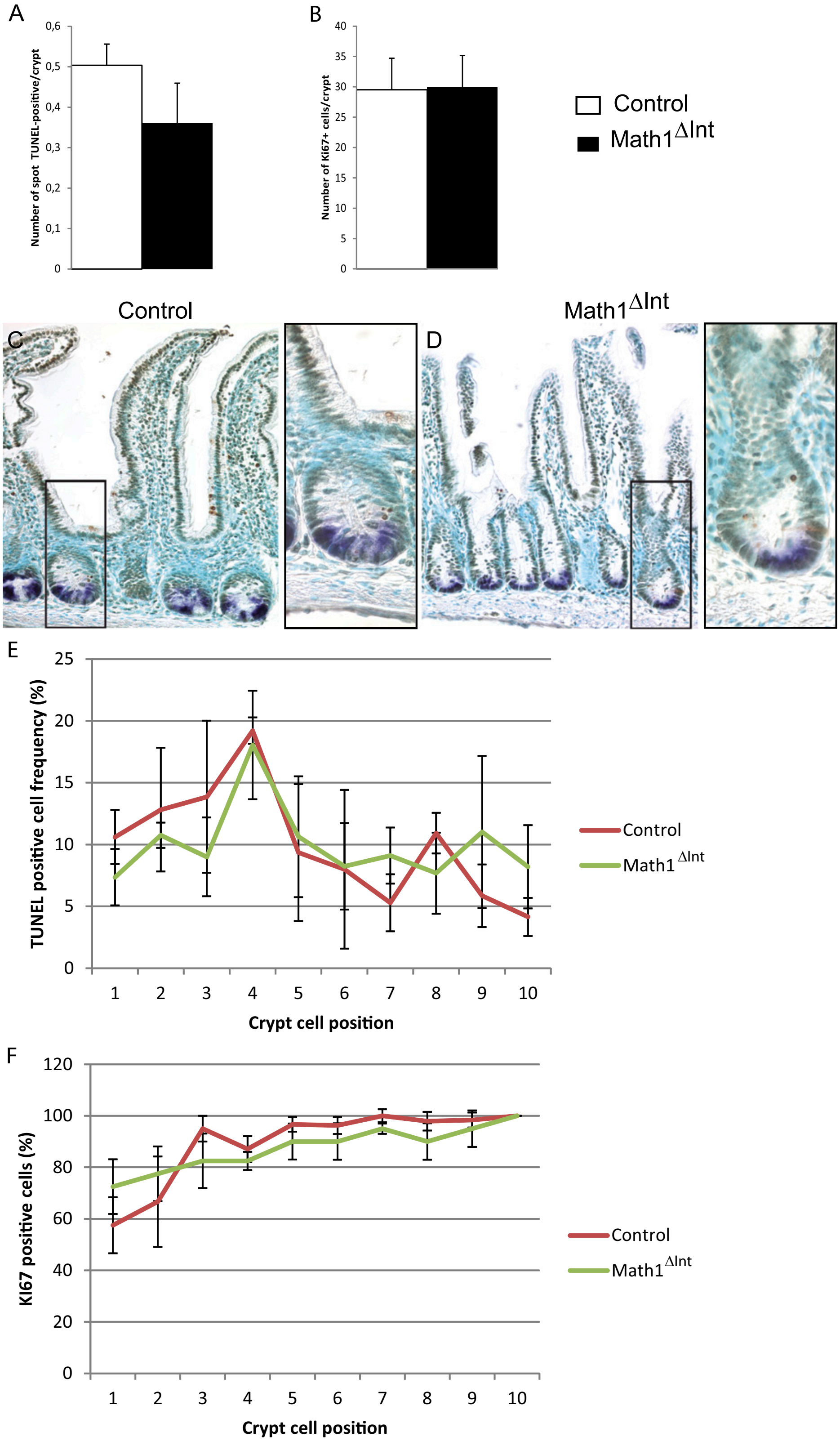


Fig. S2. Similar cell proliferation and apoptosis in Math1-deficient crypt. (*A* and *B*) Quantification of number of spot TUNEL-positive (*A*) and Ki67-positive (*B*) cells per crypt section in control and Math1 mutant mice 7 d after Tam injection. No significant differences were observed. (*C* and *D*) Representative sections of TUNEL labeling in control (*C*) and Math1 mutant (*D*) mice 7 d after Tam injection. (*E* and *F*) Frequency of TUNEL-positive cells (*E*) and percentage of Ki67 (*F*) at individual crypt cell position (from 1 to 10) in control and Math1 mutant mice 7 d after Tam injection. CBCs are localized at the base of the crypt, and the Bmi^{+} are located predominantly at four cells above the base of the crypt (+4). No significant differences were observed.

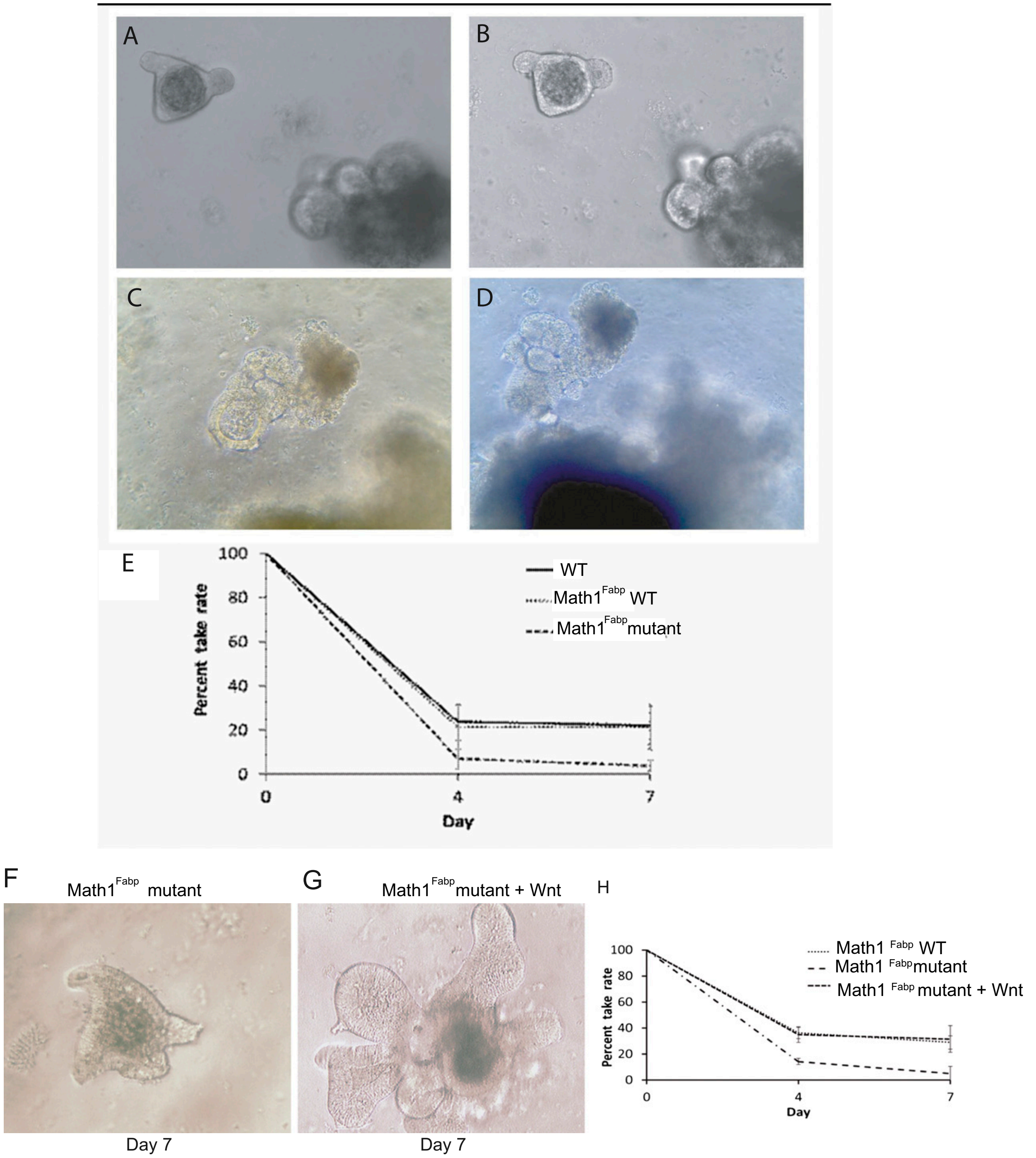


Fig. S3. Math1^{FABP} mutant organoids can survive in the presence of wild-type organoids or additional Wnt3 ligand. Culture of mosaic Math1^{FABP} crypts over a period of 2 wk. Math1^{Fabp} mutant crypts, identified by lack of Paneth cell granules, that were established near Math1^{Fabp} wild-type crypts formed nascent spheroids, which grew at a significantly slower rate compared with wild type organoids as seen at 4 d (*A*), 7 d (*B*), 12 d (*C*), and 16 d (*D*). (*E*) Frequency of organoid formation from wild-type and mutant crypts (*n* = 4 wild-type mice, 998 crypts; *n* = 4 Math1^{FABP} mice, 1,426 crypts). Crypts from Math1^{Fabp} mutant crypts were cultured in normal media (*F*) and in media supplemented with Wnt3a (*G*). (*H*) Addition of Wnt3a restored the frequency of organoid formation in Math1^{Fabp} mutant crypts to a level comparable to wild type organoids.

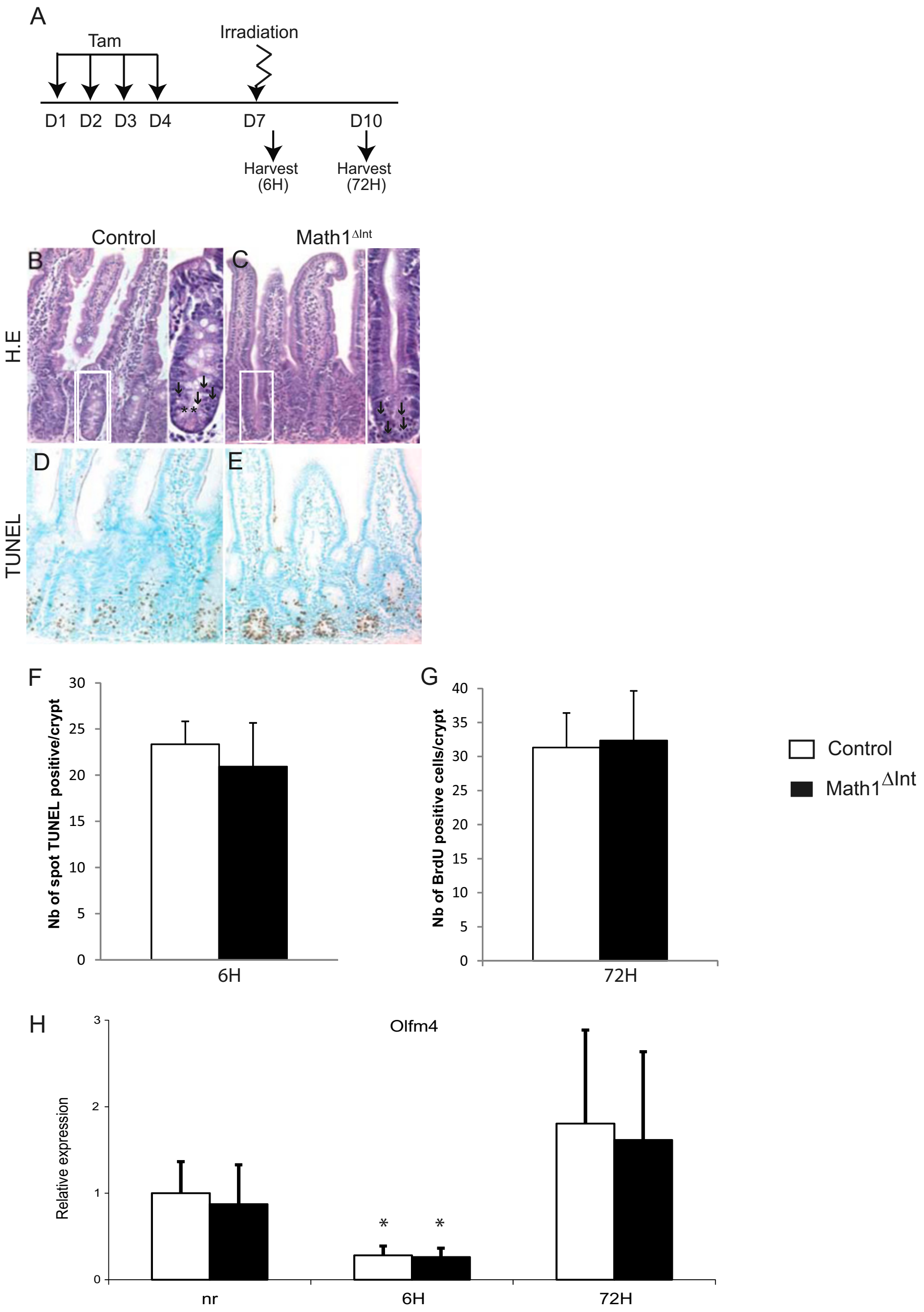
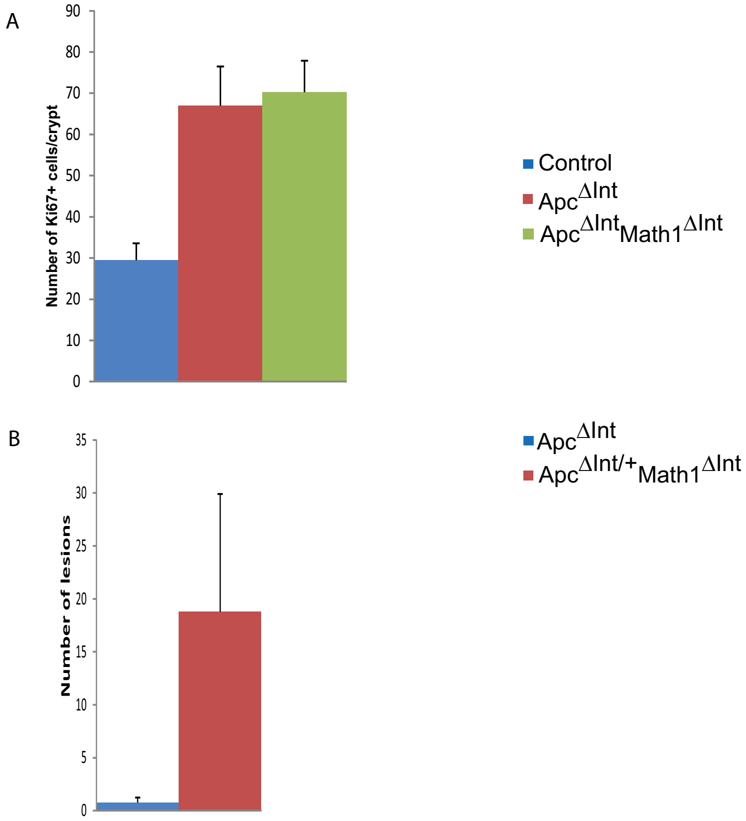


Fig. S4. Math1 deletion has no effect on intestinal crypt cell apoptosis after radiation injury. (*A*) Treatment schema: Tam was injected into control and Math1 mutant mice for 4 consecutive days before they were irradiated (10 Gy) on day 7 after the first injection. Animals were killed 6 or 72 h after irradiation. H.E. (*B* and *C*) and TUNEL staining (*D* and *E*) demonstrate that similar levels of apoptotic cells were present in control and Math1 mutant mice 6 h after irradiation. (*F* and *G*) Quantification of the number of spot TUNEL-positive per crypt section (*F*) and BrdU-positive cells (*G*), respectively, 6 and 72 h after irradiation in control and Math mutant mice. (*H*) Real-time quantitative RT-PCR analysis of Olfm4 in the small intestine of control and Math mutant mice before and after irradiation (6 and 72 h). nr, nonirradiated. Significant difference was observed between nr mice and irradiated mice at 6 h after irradiation (*$P < 0.05$).



C

	Number of dysplastic glands	Size of lesions (μm)	% of lesions
Apc$^{\Delta Int/+}$	1	72-195	100
Apc$^{\Delta Int/+}$Math1$^{\Delta Int}$	1	51-209	47
	2	124-260	32
	3	161-271	12
	4	165-297	7
	5	403-457	2

Fig. S5. Analysis of cell proliferation in Apc and Apc Math1 mutant mice. (*A*) Quantification of Ki67-positive cells in control, Apc$^{\Delta Int}$, and Apc$^{\Delta Int}$Math1$^{\Delta Int}$ 5 d after Tam injections. No significant difference between Apc$^{\Delta Int}$ and Apc$^{\Delta Int}$Math1$^{\Delta Int}$ was observed. (*B*) Quantification of intestinal lesions observed in Apc$^{\Delta Int/+}$ and Apc$^{\Delta Int/+}$Math1$^{\Delta Int}$ at 22 d after Tam injection ($P < 0.01$). (*C*) Table describing the size and the percentage of each lesions depending on the number of dysplastic glands observed in each genotype in Apc$^{\Delta Int/+}$ and Apc$^{\Delta Int/+}$Math1$^{\Delta Int}$ at 22 d after Tam injection.

Table S1. Target genes found misexpressed in small intestine of adult and young Math1$^{\Delta\text{int}}$ mice

			Fold differences	
Affymetrix ID	Symbol	Name	Adult Math1$^{\Delta\text{int}}$	Young Math1$^{\Delta\text{int}}$
Down-regulated target genes				
Secretory lineage				
1449822	Math1	Mouse atonal homolog 1	32.0	13.4
Paneth cell markers				
1449478	MMP7	Matrix metallopeptidase 7	160.4	78.8
1450631	DEFCR24	Defensin related cryptdin 24	152.8	93.5
1436857	DEFCR21	Defensin related cryptdin 21	116.7	149.2
1450709	DEFCR5	Defensin related cryptdin 5	104.7	75.4
1418282	SERPINA1	Serpin peptidase inhibitor clade A	21.7	8.8
1419204	DLL1	Delta-like 1	2.1	2.8
Goblet cell markers				
1416306	CLCA1	Chloride channel accessory 1	1369.4	410.8
1428435	MUC2	Mucin 2	837.5	185.4
1417370	TFF3	Trefoil factor 3	742.5	128.0
1426872	FCGBP	Fc fragment of IgG binding protein	467.8	325.6
1450220	SPDEF	SAM pointed domain containing ets transcription factor	11.5	7.2
1455802	AGR2	Anterior gradient homolog 2	8.4	9.0
1427119	SPINK4	Serine peptidase inhibitor Kazal type 4	1.6	87.9
Enteroendocrine cell markers				
1434137	LOC653808	Zymogen granule membrane protein	877.8	276.7
1422860	NTS	Neurotensin	17.4	24.3
1415885	CHGB	Chromogranin B	11.4	6.6
1421594	SYTL2	Synaptotagmin-like 2	11.3	8.9
1424865	PYY	Peptide YY	9.8	9.1
1418805	SCT	Secretin	7.3	7.8
1418149	CHGA	Chromogranin A	4.7	4.1
1426413	NEUROD1	Neurogenic differentiation 1	1.7	3.0
Others				
1420437	IDO1	Indoleamine 2.3-dioxygenase 1	75.5	181.8
1435939	HEPACAM2	HEPACAM family member 2	30.0	15.4
1415837	KLK2	Kallikrein-related peptidase 2	21.2	11.7
1417266	CCL6	Chemokine (C-C motif) ligand 6	19.1	11.8
1419665	NUPR1	Nuclear protein 1	13.1	5.7
1422876	CAPN9	Calpain 9	9.9	4.6
1420334	SLC12A8	Solute carrier family 12 member 8	8.6	3.1
1451498	LRRC26	Leucine rich repeat containing 26	8.4	3.3
1427063	KIAA1324	KIAA1324	7.8	3.4
1449564	TPSG1	Tryptase γ 1	7.1	2.4
1417481	RAMP1	Receptor (G protein-coupled) activity modifying protein 1	6.1	2.3
1419248	RGS2	Regulator of G protein signaling	4.5	3.2
1438676	GBP6	Guanylate binding protein family member 6	4.2	19.3
1429123	RAB27A	RAB27A	3.7	3.6
1427308	DAB1	Disabled homolog 1	3.7	2.4
1449896	MLPH	Melanophilin	3.0	2.3
1458347	TMPRSS2	Transmembrane protease serine 2	2.9	2.5
1425853	PRLR	Prolactin receptor	2.6	3.1
1434140	MCF2L	MCF.2 cell line derived transforming sequence-like	2.5	3.0
1457269	GM1123	Gene model 1123	2.4	1.6
1417985	NRARP	NOTCH-regulated ankyrin repeat protein	2.4	3.2
1420505	STXBP1	Syntaxin binding protein 1	2.2	2.0
1435043	PLCB1	Phospholipase C β 1	2.2	1.5
1425216	FFAR2	Free fatty acid receptor 2	2.1	2.0
1449556	HLA-E	Major histocompatibility complex, class I, E	2.1	4.4
1416950	TNFAIP8	Tumor necrosis factor α-induced protein 8	2.0	2.9
1421211	CIITA	Class II, major histocompatibility complex, transactivator	2.0	2.8
1441793	RNF39	Ring finger protein 39	2.0	1.6
1417898	GZMA	Granzyme A	2.0	22.3

Table S1. Cont.

Affymetrix ID	Symbol	Name	Fold differences	
			Adult Math1$^{\Delta int}$	Young Math1$^{\Delta int}$
1458591	RASEF	RAS and EF-hand domain containing	1.9	2.1
1436297	GRINA	Glutamate receptor ionotropic *N*-methyl D-aspartate-associated protein 1	1.9	2.6
1418891	RAB3D	RAB3D	1.9	1.5
1439773	LY6E	Lymphocyte antigen 6 complex, locus E	1.9	2.5
1418734	BE136769	Expressed sequence BE136769	1.8	2.6
1419295	CREB3L1	cAMP responsive element binding protein 3-like 1	1.8	1.8
1431008	HLA-B	Major histocompatibility complex, class I, B	1.8	4.3
1418240	GBP2	Guanylate binding protein 2	1.7	19.4
1453913	TAP2	Transporter 2 ATP-binding cassette subfamily B	1.7	4.2
1441850	TCN2	Transcobalamin II	1.7	1.6
1449875	H2-T10	Histocompatibility 2, T region locus 10	1.7	3.3
1450350	JDP2	Jun dimerization protein 2	1.7	3.4
1455251	ITGA1	Integrin α 1	1.6	1.8
1455566	LOC26010	Viral DNA polymerase-transactivated protein 6	1.5	2.5
1450534	HLA-C	Major histocompatibility complex, class I, C	1.5	2.4
1418392	GBP4	Guanylate binding protein 4	1.5	9.4
Up-regulated target genes				
1416930	LY6D	Lymphocyte antigen 6 complex locus D	8.7	8.8
1448894	TCAG7.1260	Aldo-keto reductase family 1 member B10-like	5.6	5.2
1451385	FAM162A	Family with sequence similarity 162 member A	5.3	3.3
1421041	GSTA5	GST α 5	4.8	13.4
1417932	IL18	Interleukin 18	4.0	5.9
1420715	PPARG	Peroxisome proliferator-activated receptor γ	2.7	1.8
1449424	PLEK2	Pleckstrin 2	2.7	1.8
1439148	PFKL	Phosphofructokinase	2.5	2.6
1420499	GCH1	GTP cyclohydrolase 1	2.3	2.7
1429299	DDAH1	Dimethylarginine dimethylaminohydrolase 1	2.2	2.3
1438376	TRIM27	Tripartite motif-containing 27	2.2	1.7
1447745	AQP4	Aquaporin 4	2.0	2.4
1450455	AKR1C12	Aldo-keto reductase family 1 member C12	2.0	3.2
1425933	NT5C2	5-Nucleotidase cytosolic II	2.0	2.5
1449005	SLC16A3	Solute carrier family 16 member 3	1.9	1.9
1447807	PLEKHH1	Pleckstrin homology domain containing family H member 1	1.9	1.9
1417122	VAV3	vav 3 guanine nucleotide exchange factor	1.8	4.2
1460351	LOC729659	S100 calcium binding protein A11 pseudogene	1.8	3.1
1435917	OCIAD2	OCIA domain containing 2	1.8	3.2
1416614	EID1	EP300 interacting inhibitor of differentiation 1	1.8	1.5
1417772	GRHPR	Glyoxylate reductase/hydroxypyruvate reductase	1.8	3.3
1420641	SQRDL	Sulfide quinone reductase-like	1.7	2.1
1417308	PKM2	Pyruvate kinase	1.7	2.2
1444632	BC064078	cDNA sequence BC064078	1.6	1.5
1448249	GPD1	Glycerol-3-phosphate dehydrogenase 1	1.6	2.4
1429764	FAM101B	Family with sequence similarity 101 member B	1.6	2.7
1449324	ERO1L	ERO1-like	1.6	1.6
1416165	RAB31	RAB31	1.5	2.2
1456769	DUSP3	Dual specificity phosphatase 3	1.5	1.5
1417293	HS6ST1	Heparan sulfate 6-*O*-sulfotransferase 1	1.5	1.7
1435536	RSC1A1	Regulatory solute carrier protein family 1 member 1	1.5	1.5
1441931	GSS	Glutathione synthetase	1.5	1.8
1422076	ACOT4	Acyl-CoA thioesterase 4	1.5	2.4
1434794	RHOF	ras homolog gene family member F	1.5	2.0