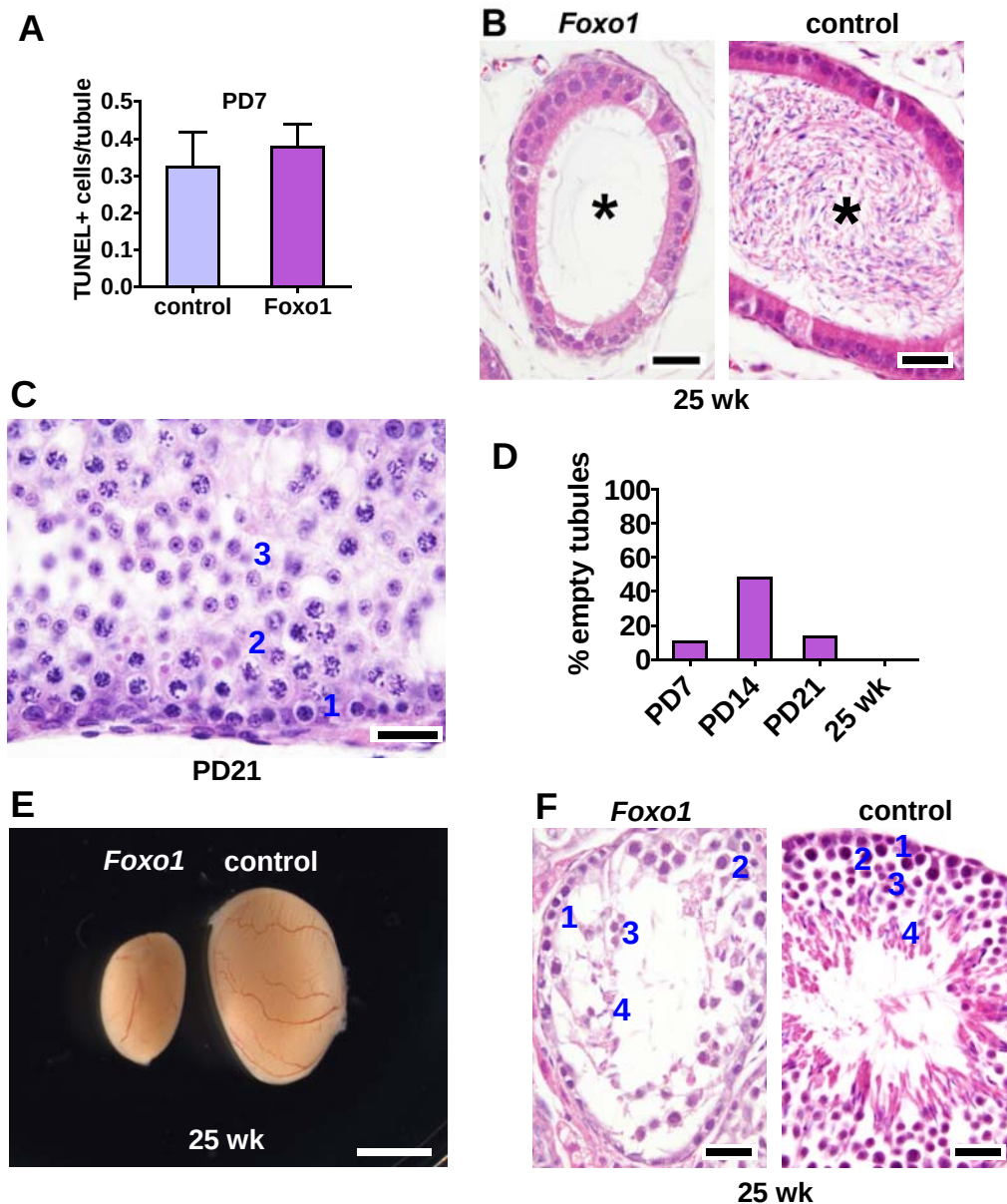
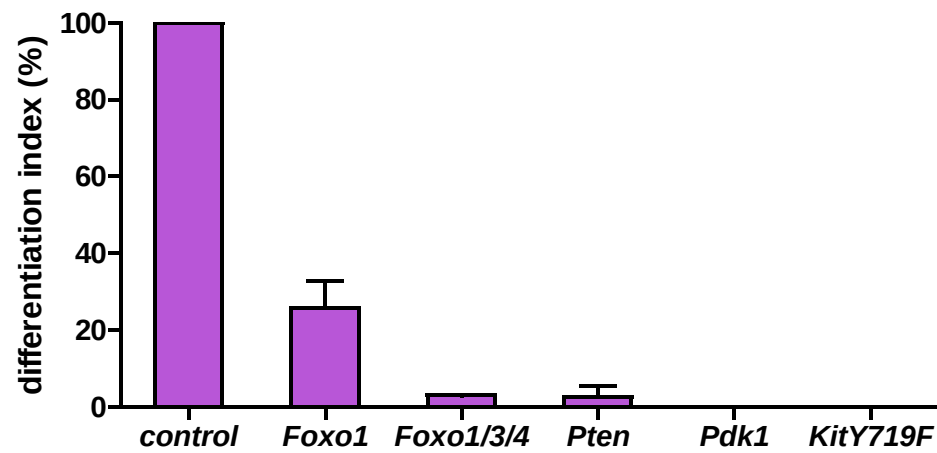
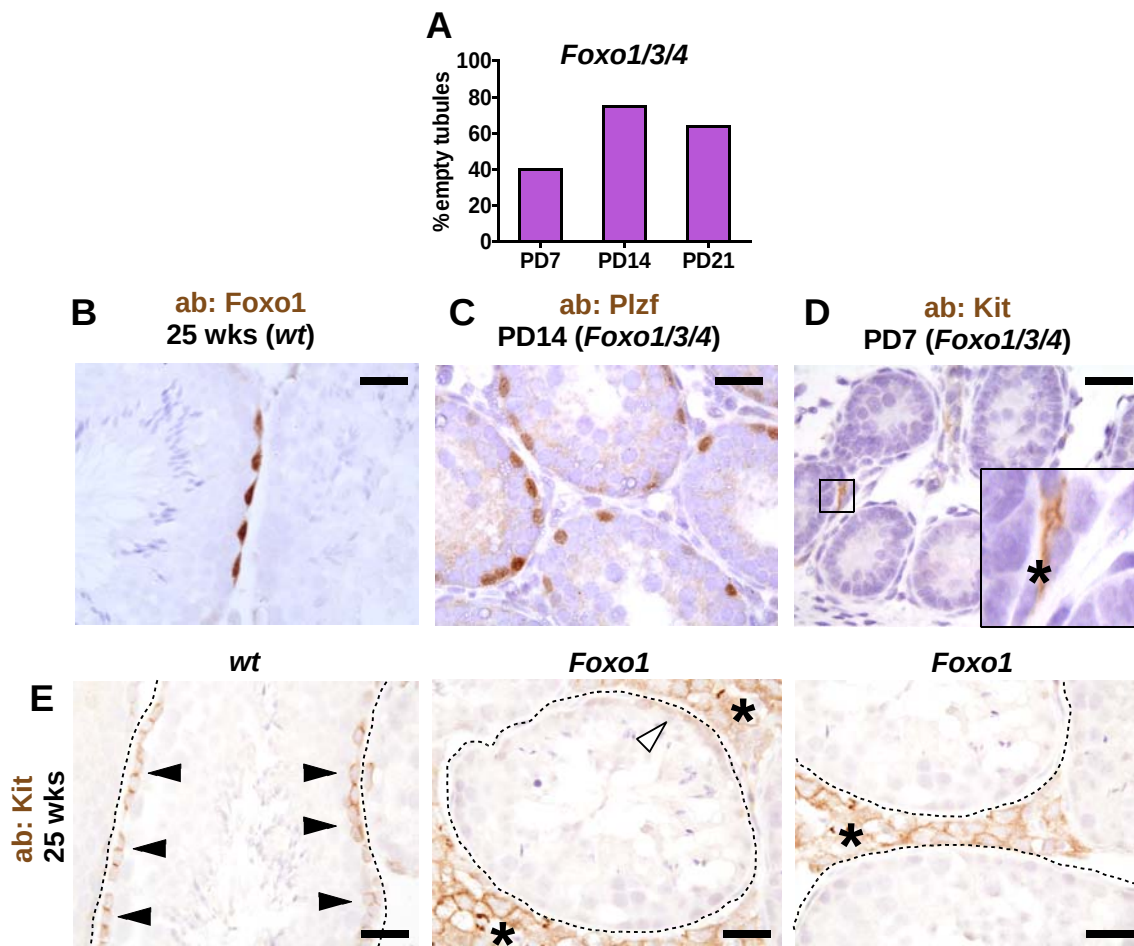




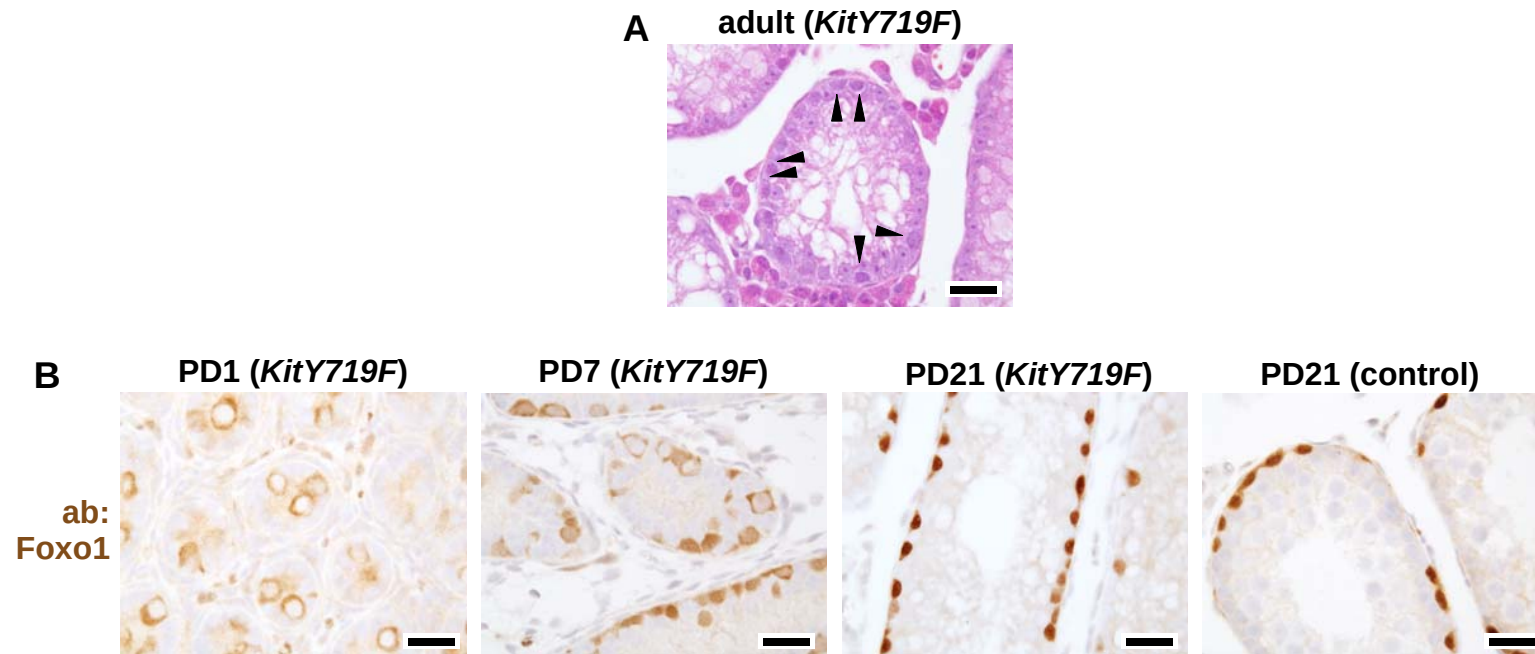
Supplemental Figure 1. Further evaluations of *Foxo1* testes. **(A)** TUNEL positive cells normalized to number of GCNA positive cells. **(B)** Tissue sections at 25 weeks of age. No sperm were present in tissue sections of epididymides (asterisks) or in epididymal caudal fluid (not shown) at 25 wks or any other age; bars=20 μ . **(C)** *Foxo1* phenotype is variable within tubules, shown is relatively unaffected tubule at PD21 with multilayer spermatogenesis including all steps normally present at PD21 including spermatogonia (1), premeiotic spermatocytes (2) and postmeiotic round spermatids (3); bar=20 μ . **(D)** Percent of empty tubules harboring no germ cells per GCNA immunostains. Controls contained no empty tubules at these timepoints. **(E)** *Foxo1* and control testes at 25 wks, bar=3 mm. **(F)** Adult (25 weeks) *Foxo1* tubules have markedly diminished spermatogenesis but all stages of spermatogenesis including spermatogonia (1), spermatocytes (2), spermatids (3), and elongated spermatozoa (4); bars=20 μ .



Supplemental Figure 2. Differentiation index (percentage of seminiferous tubules showing multilayer spermatogenesis) for each mutant in this study and wild-type controls at PD21.



Supplemental Figure 3. Additional studies of *Foxo1*, *Foxo1/3/4*, and control testes. **(A)** Empty tubules harboring no germ cells in *Foxo1/3/4* tissue sections immunostained with GCNA. Control testes contained no empty tubules at these timepoints. **(B)** *Foxo1* immunostain of *wt* adult mouse. Cell clustering (consistent with a portion of germline cyst in the plane of section) is sometimes observed. **(C)** *Plzf*⁺ and *Plzf*⁻ spermatogonia in *Foxo1/3/4* testes at PD14. **(D)** *Kit* underexpression in *Foxo1/3/4* testes at PD7. Inset shows *Kit* in Leydig cells (asterisk) as internal positive control. Bars=20 μ for **B-D**. **(E)** *Kit* underexpression in adult *Foxo1* testis (25 weeks), tubule borders highlighted by dashed lines. In *wt* control (left panel), *Kit* is prominent in subset of tubules in a stage-dependent manner (arrowheads); this is not observed in *Foxo1* testis. Middle panel shows tubule with minimal focal *Kit* expression (arrowhead); right panel shows tubules with no *Kit* expression. *Kit*⁺ Leydig cells are prominent in *Foxo1* testes (Leydig cell hyperplasia secondary to failure of spermatogenesis, asterisks). Bars=20 μ .



Supplemental Figure 4. Foxo1 expression and its subcellular localization in gonocytes and undifferentiated spermatogonia are not regulated by Kit-PI3K signalling. (**A**) H&E-stained section from adult *KitY719F* male showing failure of spermatogenic initiation manifest by absence of multilayer spermatogenesis (spermatogonial arrest). Multiple spermatogonia are present and can be distinguished from Sertoli cells by their distinct chromatin pattern and lack of prominent nucleoli (arrowheads). (**B**) Immunostaining for Foxo1 in *KitY719F* testis at various ages. A PD21 wild-type control testis is shown on the right; see Figure 3 for additional control timepoints. Bars=20 μ for **A** and **B**.

Supplemental Table 1. Expression profiling of Foxo1 vs. control testes at P04

PROBE_ID	SYMBOL	FOLD CHANGE	WT.AVG_Signal	WT.ARRAYS	WT.ARRAY_STDEV	WT.Detection Pval	WT.DIFF Pval	Null.AVG_Signal	Null.ARRAYS	Null.ARRAY_STDEV	Null.Detection Pval	Null.DIFF Pval	SEARCH_KEY	ILMN_GENE	CHROM	DEFINITION	SYNONYMS	UNIGENE_ID	ENTREZ_GENE_ID	ACCESSION
ILMN_1225956	17000GH03Rik	-2.25	613.7	3	231.401	0	1	281.3	3	44.358	0	0.00969	ILMN_215352	17000GH03Rik		PREDICTED: Mus musculus RIKEN cDNA 17000GH03 gene, transcript variant 3 (17000GH03Rik), mRNA.				
ILMN_2839313	Actg2	2.77	755	3	61.734	0	1	2089.7	3	1168.687	0	0.04824	ILMN_210831	ACTG2	6	Mus musculus actin, gamma 2, smooth muscle, enteric (Actg2), mRNA.	SMGA; Act4; ACTA3; Act-4	74174 NM_017380.3	11468 NM_009610.1	
ILMN_2764727	Actn2	-2.15	426	3	85.538	0	1	197.7	3	18.69	0	0.00001	ILMN_223487	ACTN2				NM_033268.2		
ILMN_2670038	Cdrl16	-4.02	205.4	3	39.226	0	1	826.4	3	528.229	0	0.04248	ILMN_185955	CDRL16				12556 NM_007663.2		
ILMN_3115629	CN716893	-2.24	2163.1	3	703.696	0	1	964.6	3	301.828	0	0.00671	ILMN_231406	CN716893	X	8 Mus musculus cadherin 16 (Cdh16), mRNA.			434903 NM_001033492.2	
ILMN_1250353	CN716893	-2.06	1753.8	3	685.599	0	1	853.2	3	342.059	0	0.04179	ILMN_198737	CN716893	X	Mus musculus expressed sequence CN716893 (CN716893), mRNA.			434903 NM_001033492.2	
ILMN_2733257	Col9a3	-2.22	640.5	3	132.358	0	1	289.1	3	65.578	0	0.00004	ILMN_221271	COL9A3		2 Mus musculus collagen, type IX, alpha 3 (Col9a3), mRNA.			12841 NM_009936.2	
ILMN_2593476	Dppa4	-3.13	838.7	3	85.408	0	1	268.3	3	99.066	0	0	ILMN_195610	DPPA4		16 Mus musculus developmental pluripotency associated 4 (Dppa4), transcript variant 1, mRNA.	AV000866		73693 NM_028610.2	
ILMN_2623983	Egr2	-2.78	836.2	3	39.421	0	1	301	3	59.331	0	0	ILMN_212209	EGR2				NM_010118.1		
ILMN_1215713	Egr4	-3.44	1541.8	3	183.598	0	1	448.4	3	335.577	0	0	ILMN_213894	EGR4		6 Mus musculus early growth response 4 (Egr4), mRNA.	NGF1-C; NGF1-C; pAT133; NGF1C		13656 NM_020596.2	
ILMN_2674620	Ela2a	-2.07	437.5	3	42.786	0	1	211	3	19.123	0	0	ILMN_216734	ELA2A		4 Mus musculus elastase 2A (Ela2a), mRNA.	Ela-2; Ela2		13706 NM_007919.2	
ILMN_1228515	Fbxo11	-2.12	333.2	3	66.606	0	1	157	3	32.74	0	0.00005	ILMN_218811	FBXO11		6 Mus musculus F-box protein 41 (Fbxo41), mRNA.	9630017H13; D6Ertd538e		330369 NM_001001160.2	
ILMN_1242370	Fgl1	-3.47	535.5	3	175.375	0	1	154.4	3	2.886	0	0.00018	ILMN_224055	FG11		8 Mus musculus fibrinogen-like protein 1 (Fgl1), mRNA.	Mfire1; MGC17822		234199 NM_145594.1	
ILMN_2897167	Fgl1	-2.03	276.3	3	60.532	0	1	136.3	3	4.504	0	0	ILMN_261972	FG11		3 Mus musculus forkhead box O1 (Foxo1), mRNA.	Fkhr1; FKHR; A876417; Ash; Foxo1a		56458 NM_019739.2	
ILMN_2656498	Foxo1*	-2.09	375.7	3	12.504	0	1	180	3	23.164	0	0	ILMN_194758	FOXO1				NM_019739		
ILMN_2509340	Foxo1*	3.71	140.3	3	11.264	0	1	520.5	3	37.683	0	0	ILMN_194758	FOXO1						
ILMN_2612283	Gata2	2.24	298.2	3	19.38	0	1	669	3	207.076	0	0.00207	ILMN_211079	GATA2		6 Mus musculus GATA binding protein 2 (Gata2), mRNA.	MGC129339; Gata-2		14461 NM_008090.4	
ILMN_3006804	Igf2bp1	-2.09	632	3	99.168	0	1	302.9	3	75.422	0	0.00001	ILMN_226669	IGF2BP1		11 Mus musculus insulin-like growth factor 2 mRNA binding protein 1 (Igf2bp1), mRNA.	D11Moh45; A1W549074; CRD-BP; Zbp1; Neilsen; IMP-1; Crdbp; D030026A21Rik; AL024068		140486 NM_009951.3	
ILMN_2930897	Igfbp2	2.73	273.4	3	36.739	0	1	747.2	3	302.472	0	0.00712	ILMN_222264	IGFBP2		1 Mus musculus insulin-like growth factor binding protein 2 (Igfbp2), mRNA.	Igfbp-2; A1Z55832		16008 NM_008342.2	
ILMN_1236788	Igfbp2	2.77	255.4	3	32.98	0	1	708.6	3	380.87	0	0.04546	ILMN_222264	IGFBP2		1 Mus musculus insulin-like growth factor binding protein 2 (Igfbp2), mRNA.	Igfbp-2; A1Z55832		16008 NM_008342.2	
ILMN_2722616	Krt14	3.11	180.8	3	47.701	0	1	562.8	3	271.811	0	0.01664	ILMN_224079	KRT14		11 Mus musculus keratin 14 (Krt14), mRNA.	Krt1-14; Krt-11; K14; A1626930		16664 NM_016958.1	
ILMN_1225672	Lhx1	-2.14	408.1	3	91.488	0	1	191	3	34.906	0	0.00014	ILMN_193570	LHX1		10 Mus musculus monoxygenase, DBH-like 1 (Mxd1), mRNA.	MNCb-3303; Z23040ZNO8Rik		NM_008498	
ILMN_2977492	Mxd1	2.04	145.5	3	14.352	0	1	297.1	3	15.801	0	0	ILMN_221576	MXD1				59012 NM_021509.3		
ILMN_1252173	Nudt6	-2.20	342.5	3	12.534	0	1	155.4	3	7.051	0	0	ILMN_210707	NUDT6		3 Mus musculus nudix (nucleoside diphosphate-linked moiety X)-type motif 6 (Nudt6), mRNA.	MGC27904; Aulgf2b		229228 NM_153561.2	
ILMN_2731331	Pkd2l1	-2.24	501.7	3	84.712	0	1	224.1	3	45.208	0	0	ILMN_221125	PKD2L1		19 Mus musculus polycystic kidney disease 2-like 1 (Pkd2l1), mRNA.	PCL; BC046386; BB30002B15; Pkd; PKD2L1; TRPP3		329064 NM_181422.2	
ILMN_2947559	Rbm35a	-2.34	1526.7	3	91.828	0	1	653.4	3	79.467	0	0	ILMN_210806	RBM35A		4 Mus musculus RNA binding motif protein 35A (Rbm35a), mRNA.	MGC25805; Z210008MA09Rik; A630065D16; BC031468		207920 NM_194055.1	
ILMN_1236477	Ret	-3.24	455.9	3	64.935	0	1	140.9	3	40.102	0	0	ILMN_186571	RET		6 Mus musculus ret proto-oncogene (Ret), transcript variant 4, mRNA.	PTC; RETS1; RETY; c-RET		19713 NM_001080780.1	
ILMN_3124235	Sall4	-3.26	964.3	3	341.119	0	1	296.2	3	159.859	0	0.00214	ILMN_232025	SALL4		2 Mus musculus sal-like 4 (Drosophila) (Sall4), transcript variant b, mRNA.	C130011P20Rik; A1W536104; Tex20; AA407717; C78563; AL022809; 5730441M18Rik; C78083		99377 NM_201395.2	
ILMN_1246800	Serpina3n	2.71	217.6	3	41.539	0	1	589.5	3	169.812	0	0.00024	ILMN_222516	SERPINA3N		12 Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 3N (Serpina3n), mRNA.	Sp12/eb-4; Sp12-2; Sp12.2		20716 NM_009252.2	
ILMN_1258935	Sult4a1	-2.13	338.1	3	62.818	0	1	158.9	3	24.031	0	0.00001	ILMN_216683	SULT4A1		15 Mus musculus sulfotransferase family 4A, member 1 (Sult4a1), mRNA.	2400007A17Rik; Sultx3; A1B53543; BR-STL-1		29859 NM_013873.3	
ILMN_1231513	Tacstd2	2.82	133.2	3	12.952	0.00108	1	375.1	3	159.15	0	0.00892	ILMN_218291	TACSTD2		6 Mus musculus tumor-associated calcium signal transducer 2 (Tacstd2), mRNA.	EGP-1; MGC141612; TROP2; MGC141611; GA733-1; CB0403; Ly97		56753 NM_020947.3	
ILMN_2985612	Tcl1	-3.86	679.6	3	86.926	0	1	175.9	3	58.718	0	0	ILMN_213546	TCL1		12 Mus musculus T-cell lymphoma breakpoint 1 (Tcl1), mRNA.			21432 NM_009337.2	
ILMN_2615917	Tex19	-2.35	1755.7	3	708.022	0	1	747.2	3	283.591	0	0.02203	ILMN_211439	TEX19		11 Mus musculus testis expressed gene 19 (Tex19), mRNA.	2410081M02Rik		73679 NM_028602.2	
ILMN_2502290	Trim71	-2.26	418.6	3	103.877	0	1	185.3	3	55.064	0	0.00064	ILMN_193987	TRIM71		9 Mus musculus tripartite motif-containing 71 (Trim71), mRNA.	Gm1127; Iin-41; Z610206G21Rik; Ripply2; min-41; Lin41		636931 NM_001042503.1	
ILMN_2467287	Tuba3a	-2.03	3446.7	3	976	0	1	1696.4	3	411.413	0	0.00421	ILMN_190114	TUBA3A		6 Mus musculus tubulin, alpha 3A (Tuba3a), mRNA.	M[a]3; A528779; Tuba3; MGC107295		22144 NM_009446.2	

*Note: Foxo1 probe ILMN_2656498 maps to the Foxo1 cDNA and thus is a bona fide reporter of Foxo1 mRNA expression. Probe ILMN_2509340 maps to a region 3' of the gene and canonical Foxo1 exons. The increase in its signal may reflect aberrant splicing due to deletion of an upstream exon in the Foxo1 conditional knockout.