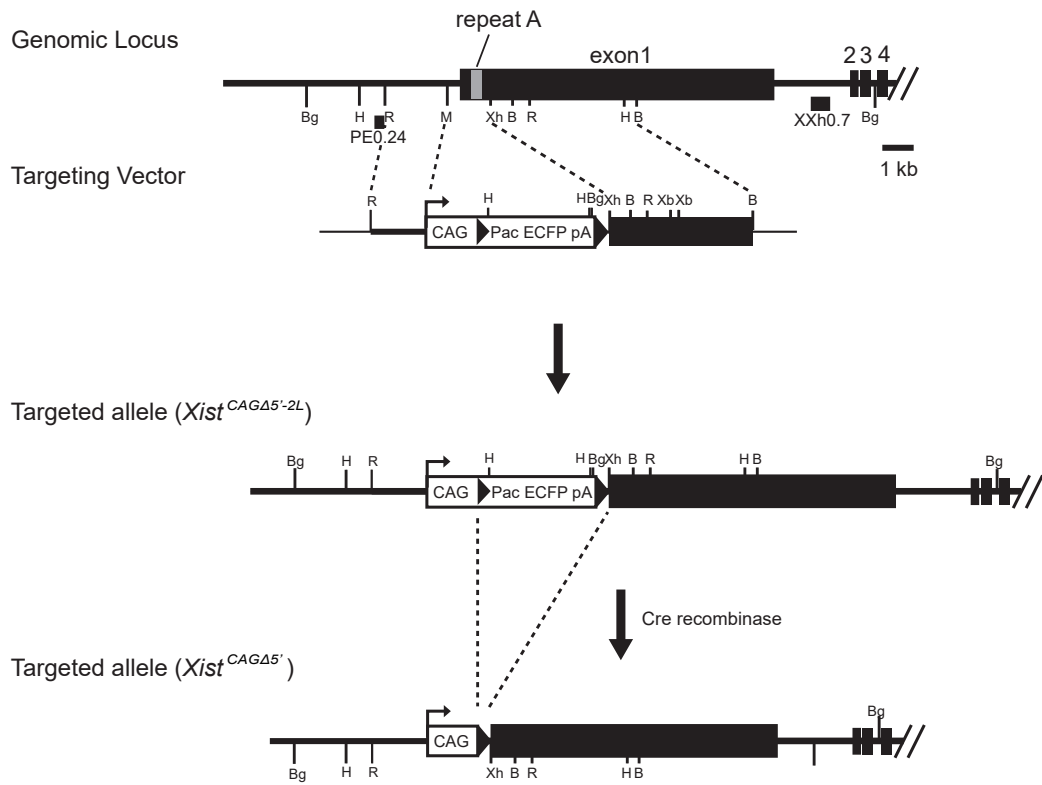
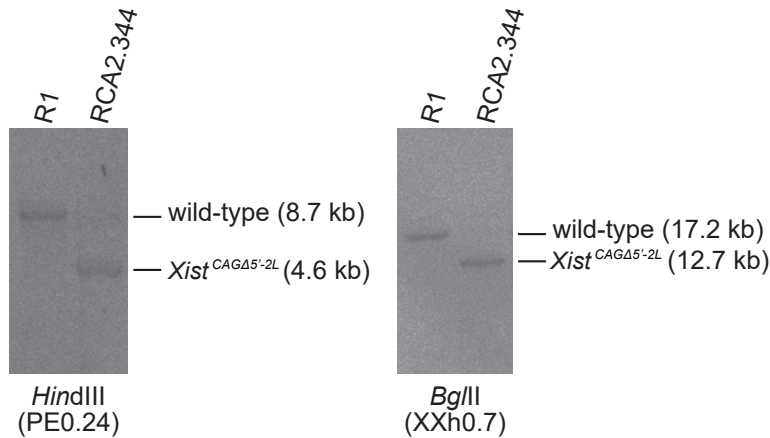


SUPPLEMENTAL FIGURES

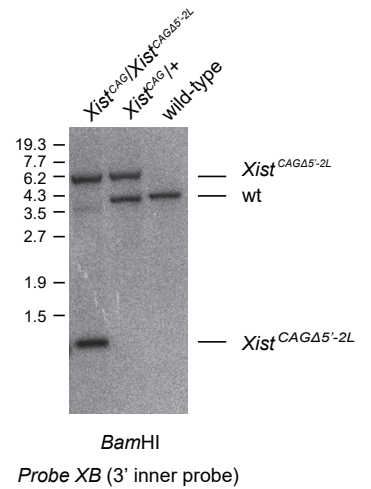
A



B



C



D

XX x [X <sup>CAGΔ5'-2L</sup> Y;Tg(Pgk2-cre)] (3 weeks)			
	XX <sup>CAGΔ5'</sup>	XY	total
No. pups	0 (0 %)	141 (100 %)	141
36 litters (3.9 pups/litter)			

Fig. S1. Sakata & Nagao et al.

**Fig. S1. Targeting scheme for generating the *Xist*<sup>CAG $\Delta$ 5'</sup> allele**

**(A)** Genomic fragment containing the endogenous *Xist* promoter and the 5' region of exon 1 (-912 nt) was replaced with a CAG-Pac ECFP-pA cassette to produce the *Xist*<sup>CAG $\Delta$ 5'-2L</sup> allele. In the presence of cre recombinase, the *Xist*<sup>CAG $\Delta$ 5'-2L</sup> allele is converted into *Xist*<sup>CAG $\Delta$ 5'</sup>.

**(B)** Homologous recombination was confirmed by Southern blotting. RCA2.344 harbored the correct targeting event. Genomic DNA of RCA2.344 and parental R1 was digested with either *Hind*III (left) or *Bgl*II (right), transferred onto a nylon membrane, and probed with either PE0.24 (left) or XXh0.7 (right) (Sado, *et al*, 2005).

**(C)** Germline transmission of *Xist*<sup>CAG $\Delta$ 5'-2L</sup> was confirmed by Southern blotting. Southern blot containing tail DNA digested with *Xba*I was probed with XB (Ohhata, *et al*, 2008).

**(D)** Selective loss of female embryos carrying the paternal *Xist*<sup>CAG $\Delta$ 5'</sup> allele. No female pups were born upon paternal transmission of *Xist*<sup>CAG $\Delta$ 5'</sup>, strongly suggesting that imprinted X-inactivation was compromised in female embryos.

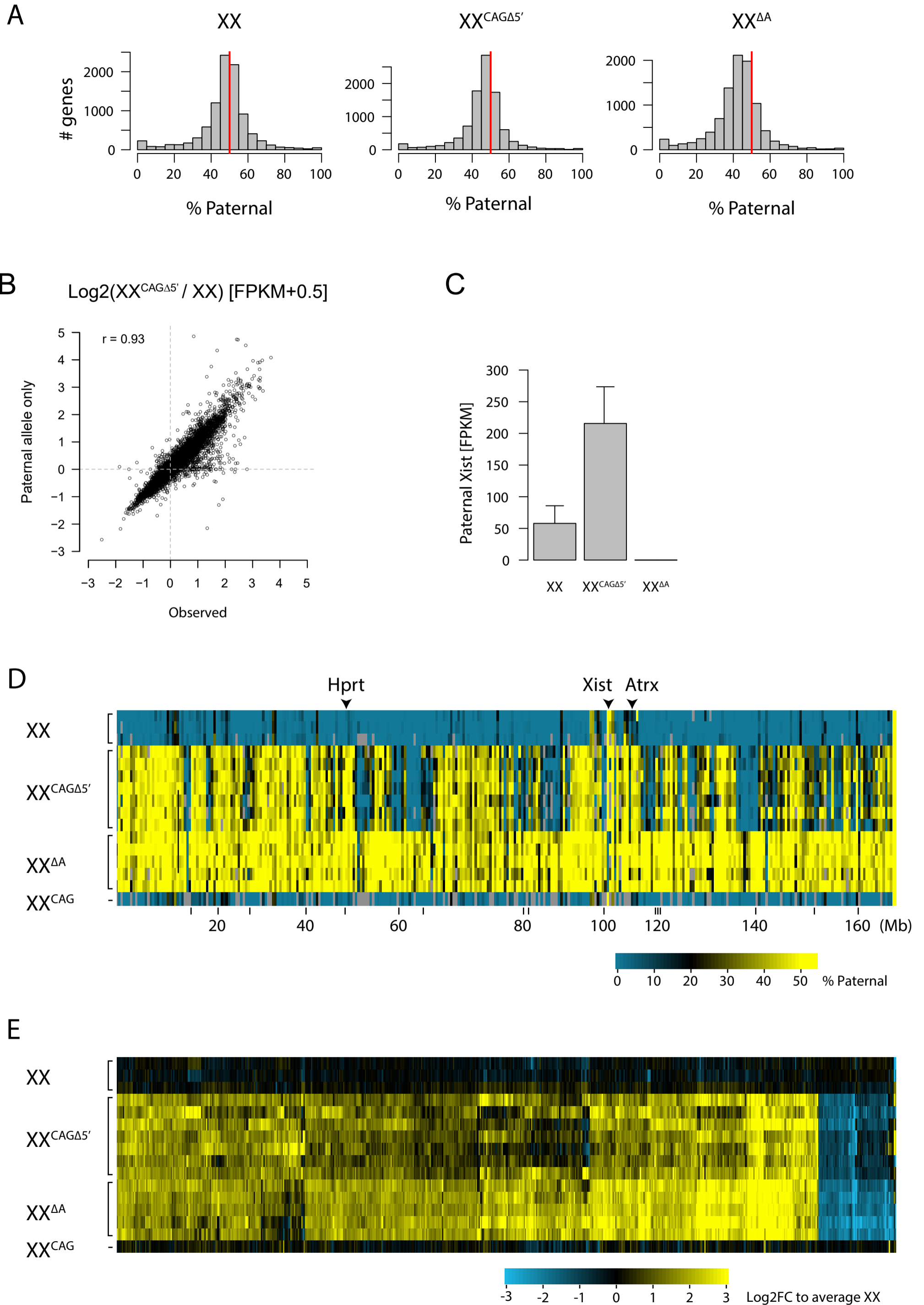


Fig. S2. Sakata & Nagao et al.

**Fig. S2. Expression profile of X-linked and autosomal genes in  $XX^{CAG^{\Delta 5'}}$  and  $XX^{\Delta A}$  in comparison with  $XX$ .**

- (A), (B) Most of the autosomal genes are biallelically expressed in the wild-type,  $XX^{CAG^{\Delta 5'}}$  and  $XX^{\Delta A}$  trophoblast. (A) Given that the mode of % paternal distribution among autosomal genes is slightly below 50% in  $XX^{CAG^{\Delta 5'}}$  and  $XX^{\Delta A}$ , it is likely that the maternal decidual tissues are contaminated in the trophoblast of both types of mutants. Since the majority of autosomal genes are expected to be equally expressed both among tissues and between alleles, we estimated that proportion of the maternal contamination in  $XX$ ,  $XX^{CAG^{\Delta 5'}}$  and  $XX^{\Delta A}$  are 0%, 5.2%, and 11.2%, respectively. (B) However, when the expression of the paternal copies, which could be identified only in the trophoblast, is extracted and plotted against the observed overall expression ratios of autosomal genes in  $XX^{CAG^{\Delta 5'}}$  relative to  $XX$ , the majority of misregulated genes exhibit a similar trend of changes as shown in Fig. 4F. This indicates that such maternal contamination would not affect our conclusion.
- (C) Expression levels of paternal *Xist* in each genotype are plotted as mean with standard deviation.
- (D) Comparison of % paternal reads of the 319 genes in all the individual trophoblast analyzed with their position along the X chromosome.
- (E) Heat map comparing the expression levels of the 1,277 autosomal genes shown in red in Figure 4F. Genes were aligned so that those show similar kinetics of the changes in their expression levels.

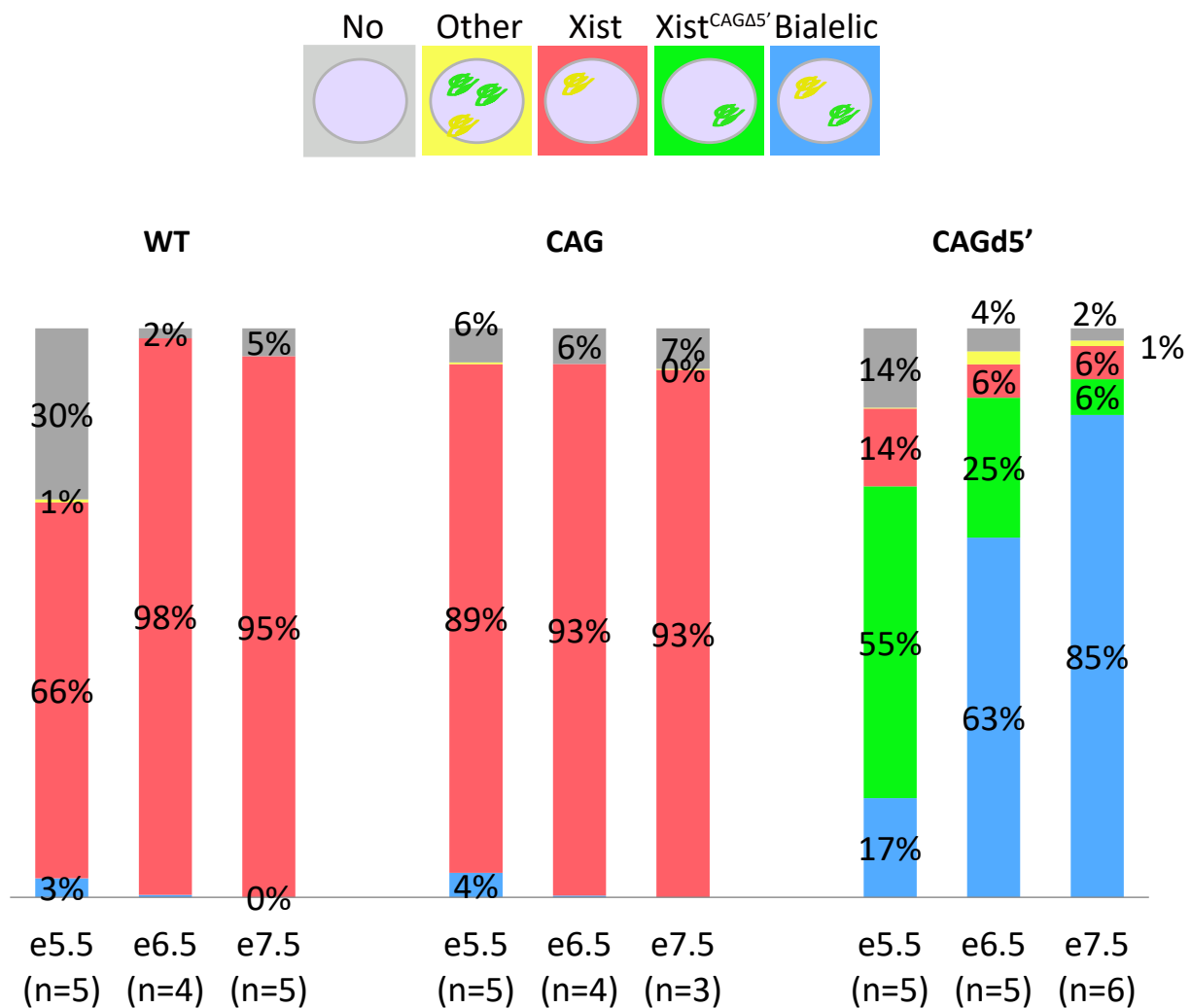


Fig. S3. Sakata &amp; Nagao et al.

**Fig. S3. Comparison of expression patterns of *Xist* in the embryonic tissue among respective genotypes at early postimplantation stages.**

Cells in the distal part of the embryo recovered from E5.5 to E7.5 were classified according to their expression patterns of *Xist* in respective genotype. While cells expressing either wild-type or mutated *Xist* RNA are both observed in XX<sup>CAGΔ5'</sup>, the population of those cells with biallelic expression increases over time. One reason why the prevalence of cells with a single mutant *Xist* cloud is higher than that of cells with a single wild-type *Xist* cloud could be ascribed to the fact that we did not remove the visceral endoderm, in which the mutated *Xist* allele is exclusively expressed due to imprinted X inactivation, from the distal part of the embryos enriched with the epiblast. Each Color of bar graphs matches that of rectangles showing respective patterns of *Xist* expression. Yellow *Xist* cloud, wild-type *Xist* RNA; green *Xist* cloud, *Xist*<sup>CAGΔ5'</sup> RNA; n, the number of embryos examined.

Table S1. Overrepresented GO terms for autosomal up-regulated genes ( $\geq 3$ -fold with significance)

GOID	Term	p-value	Benjamini
GO:0042127	regulation of cell proliferation	5.19E-18	3.11E-14
GO:0070887	cellular response to chemical stimulus	2.02E-17	6.06E-14
GO:0071310	cellular response to organic substance	5.43E-17	1.08E-13
GO:0048514	blood vessel morphogenesis	8.62E-17	1.66E-13
GO:0001568	blood vessel development	1.47E-16	1.33E-13
GO:0009966	regulation of signal transduction	2.05E-16	2.22E-13
GO:0040012	regulation of locomotion	3.72E-16	2.85E-13
GO:2000026	regulation of multicellular organismal development	3.87E-16	2.49E-13
GO:0030334	regulation of cell migration	4.16E-16	2.95E-13
GO:2000145	regulation of cell motility	4.18E-16	2.66E-13
GO:0001944	vasculature development	5.58E-16	3.02E-13
GO:0010033	response to organic substance	5.65E-16	2.77E-13
GO:0007166	cell surface receptor signaling pathway	1.98E-15	9.20E-13
GO:0051270	regulation of cellular component movement	2.01E-15	8.55E-13
GO:0051241	negative regulation of multicellular organismal process	2.73E-15	1.11E-12
GO:0022603	regulation of anatomical structure morphogenesis	2.84E-15	1.08E-12
GO:0051240	positive regulation of multicellular organismal process	5.06E-15	1.80E-12
GO:0048870	cell motility	1.32E-14	4.40E-12
GO:0051674	localization of cell	1.32E-14	4.40E-12
GO:0009605	response to external stimulus	1.50E-14	4.72E-12
GO:0008219	cell death	1.61E-14	4.82E-12
GO:0010646	regulation of cell communication	2.44E-14	6.96E-12
GO:0016477	cell migration	2.50E-14	6.80E-12
GO:0012501	programmed cell death	2.83E-14	7.37E-12
GO:0040011	locomotion	2.92E-14	7.29E-12
GO:0042981	regulation of apoptotic process	4.08E-14	9.76E-12
GO:0023051	regulation of signaling	4.82E-14	1.11E-11
GO:0048584	positive regulation of response to stimulus	6.16E-14	1.37E-11
GO:0043067	regulation of programmed cell death	7.49E-14	1.60E-11
GO:0007167	enzyme linked receptor protein signaling pathway	7.95E-14	1.64E-11
GO:0045597	positive regulation of cell differentiation	9.31E-14	1.86E-11
GO:0072359	circulatory system development	9.70E-14	1.87E-11
GO:0072358	cardiovascular system development	9.70E-14	1.87E-11
GO:0008283	cell proliferation	1.01E-13	1.89E-11
GO:0035556	intracellular signal transduction	1.17E-13	2.12E-11
GO:0045595	regulation of cell differentiation	1.38E-13	2.43E-11
GO:0010941	regulation of cell death	2.23E-13	3.82E-11
GO:0006915	apoptotic process	2.75E-13	4.57E-11
GO:0001525	angiogenesis	4.00E-13	6.48E-11
GO:0051093	negative regulation of developmental process	9.17E-13	1.45E-10
GO:0009967	positive regulation of signal transduction	2.47E-12	3.79E-10
GO:0048585	negative regulation of response to stimulus	3.59E-12	5.37E-10
GO:0048646	anatomical structure formation involved in morphogenesis	4.02E-12	5.88E-10
GO:0006952	defense response	8.19E-12	1.17E-09
GO:1902531	regulation of intracellular signal transduction	1.25E-11	1.74E-09
GO:0034097	response to cytokine	2.04E-11	2.77E-09
GO:0051094	positive regulation of developmental process	2.59E-11	3.44E-09
GO:0006928	movement of cell or subcellular component	2.83E-11	3.69E-09
GO:1901342	regulation of vasculature development	3.63E-11	4.63E-09
GO:2000147	positive regulation of cell motility	3.95E-11	4.93E-09
GO:0030335	positive regulation of cell migration	4.93E-11	6.02E-09

GO:0010942	positive regulation of cell death	5.47E-11	6.56E-09
GO:0043068	positive regulation of programmed cell death	6.23E-11	7.31E-09
GO:0009887	organ morphogenesis	6.81E-11	7.84E-09
GO:0051272	positive regulation of cellular component movement	8.50E-11	9.60E-09
GO:0023056	positive regulation of signaling	8.99E-11	9.97E-09
GO:0040017	positive regulation of locomotion	9.12E-11	9.93E-09
GO:0048468	cell development	1.24E-10	1.33E-08
GO:0008285	negative regulation of cell proliferation	1.31E-10	1.37E-08
GO:0009611	response to wounding	1.40E-10	1.44E-08
GO:0043065	positive regulation of apoptotic process	1.57E-10	1.59E-08
GO:0010647	positive regulation of cell communication	1.66E-10	1.66E-08
GO:0040007	growth	2.17E-10	2.13E-08
GO:0009628	response to abiotic stimulus	2.23E-10	2.16E-08
GO:0042060	wound healing	5.52E-10	5.25E-08
GO:0035295	tube development	6.27E-10	5.87E-08
GO:0001816	cytokine production	6.41E-10	5.91E-08
GO:0009719	response to endogenous stimulus	1.17E-09	1.06E-07
GO:0001817	regulation of cytokine production	1.19E-09	1.06E-07
GO:0006954	inflammatory response	1.21E-09	1.06E-07
GO:0009968	negative regulation of signal transduction	1.32E-09	1.15E-07
GO:0071363	cellular response to growth factor stimulus	1.44E-09	1.23E-07
GO:0045765	regulation of angiogenesis	1.73E-09	1.46E-07
GO:0030198	extracellular matrix organization	2.33E-09	1.93E-07
GO:0043062	extracellular structure organization	2.56E-09	2.10E-07
GO:0045596	negative regulation of cell differentiation	3.62E-09	2.93E-07
GO:0070848	response to growth factor	4.80E-09	3.83E-07
GO:1901343	negative regulation of vasculature development	6.31E-09	4.97E-07
GO:1901700	response to oxygen-containing compound	6.74E-09	5.24E-07
GO:0032101	regulation of response to external stimulus	8.55E-09	6.56E-07
GO:0032963	collagen metabolic process	1.01E-08	7.69E-07
GO:0044259	multicellular organismal macromolecule metabolic process	1.21E-08	9.09E-07
GO:0071345	cellular response to cytokine stimulus	1.37E-08	1.01E-06
GO:0051128	regulation of cellular component organization	1.40E-08	1.02E-06
GO:0010648	negative regulation of cell communication	1.41E-08	1.01E-06
GO:0001501	skeletal system development	1.45E-08	1.03E-06
GO:0001558	regulation of cell growth	1.53E-08	1.08E-06
GO:0023057	negative regulation of signaling	1.62E-08	1.13E-06
GO:0002684	positive regulation of immune system process	1.72E-08	1.19E-06
GO:0071495	cellular response to endogenous stimulus	2.03E-08	1.38E-06
GO:0060429	epithelium development	2.22E-08	1.50E-06
GO:0002682	regulation of immune system process	2.23E-08	1.49E-06
GO:0008284	positive regulation of cell proliferation	2.25E-08	1.48E-06
GO:0033993	response to lipid	2.76E-08	1.80E-06
GO:0016049	cell growth	2.96E-08	1.91E-06
GO:0048732	gland development	2.99E-08	1.91E-06
GO:1902533	positive regulation of intracellular signal transduction	3.58E-08	2.26E-06
GO:0006468	protein phosphorylation	3.94E-08	2.46E-06
GO:0007178	transmembrane receptor protein serine/threonine kinase signaling pathway	4.06E-08	2.51E-06
GO:0001932	regulation of protein phosphorylation	4.39E-08	2.68E-06
GO:0030855	epithelial cell differentiation	4.84E-08	2.93E-06
GO:0001667	ameboidal-type cell migration	5.65E-08	3.39E-06
GO:0061448	connective tissue development	5.97E-08	3.54E-06
GO:0030155	regulation of cell adhesion	7.05E-08	4.14E-06

GO:0042325	regulation of phosphorylation	8.13E-08	4.73E-06
GO:0022610	biological adhesion	8.33E-08	4.80E-06
GO:0051130	positive regulation of cellular component organization	8.42E-08	4.80E-06
GO:0031325	positive regulation of cellular metabolic process	9.76E-08	5.51E-06
GO:0060284	regulation of cell development	1.02E-07	5.69E-06
GO:0040008	regulation of growth	1.03E-07	5.71E-06
GO:0048608	reproductive structure development	1.21E-07	6.67E-06
GO:0051174	regulation of phosphorus metabolic process	1.33E-07	7.23E-06
GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	1.47E-07	7.95E-06
GO:0061458	reproductive system development	1.59E-07	8.50E-06
GO:0001655	urogenital system development	1.62E-07	8.57E-06
GO:0000165	MAPK cascade	1.74E-07	9.13E-06
GO:0010720	positive regulation of cell development	1.98E-07	1.03E-05
GO:0023014	signal transduction by protein phosphorylation	2.10E-07	1.08E-05
GO:0009607	response to biotic stimulus	2.24E-07	1.14E-05
GO:0032964	collagen biosynthetic process	2.52E-07	1.28E-05
GO:0007155	cell adhesion	2.59E-07	1.30E-05
GO:0019220	regulation of phosphate metabolic process	2.59E-07	1.29E-05
GO:0014070	response to organic cyclic compound	2.85E-07	1.41E-05
GO:0044236	multicellular organism metabolic process	3.06E-07	1.50E-05
GO:0043066	negative regulation of apoptotic process	3.09E-07	1.50E-05
GO:0002685	regulation of leukocyte migration	3.48E-07	1.68E-05
GO:0072001	renal system development	3.88E-07	1.86E-05
GO:0090287	regulation of cellular response to growth factor stimulus	3.96E-07	1.88E-05
GO:0001822	kidney development	4.02E-07	1.90E-05
GO:0051707	response to other organism	4.31E-07	2.02E-05
GO:0043207	response to external biotic stimulus	4.45E-07	2.06E-05
GO:0043069	negative regulation of programmed cell death	4.99E-07	2.30E-05
GO:0032835	glomerulus development	5.03E-07	2.30E-05
GO:0048762	mesenchymal cell differentiation	5.48E-07	2.49E-05
GO:0006955	immune response	6.18E-07	2.78E-05
GO:0009893	positive regulation of metabolic process	6.26E-07	2.80E-05
GO:0030323	respiratory tube development	7.05E-07	3.13E-05
GO:0072593	reactive oxygen species metabolic process	7.63E-07	3.36E-05
GO:0000904	cell morphogenesis involved in differentiation	8.00E-07	3.50E-05
GO:0010632	regulation of epithelial cell migration	8.11E-07	3.52E-05
GO:0001818	negative regulation of cytokine production	8.46E-07	3.64E-05
GO:0032268	regulation of cellular protein metabolic process	8.66E-07	3.71E-05
GO:0060548	negative regulation of cell death	9.28E-07	3.94E-05
GO:0051246	regulation of protein metabolic process	9.67E-07	4.08E-05
GO:2000181	negative regulation of blood vessel morphogenesis	1.01E-06	4.24E-05
GO:0060485	mesenchyme development	1.04E-06	4.34E-05
GO:0003007	heart morphogenesis	1.30E-06	5.36E-05
GO:0043408	regulation of MAPK cascade	1.48E-06	6.05E-05
GO:0002252	immune effector process	1.64E-06	6.67E-05
GO:0035270	endocrine system development	1.74E-06	7.03E-05
GO:0007492	endoderm development	1.76E-06	7.06E-05
GO:0010604	positive regulation of macromolecule metabolic process	1.95E-06	7.77E-05
GO:0048729	tissue morphogenesis	2.00E-06	7.92E-05
GO:0031324	negative regulation of cellular metabolic process	2.04E-06	8.06E-05
GO:0001934	positive regulation of protein phosphorylation	2.13E-06	8.33E-05
GO:0030324	lung development	2.14E-06	8.34E-05
GO:0002009	morphogenesis of an epithelium	2.32E-06	8.97E-05



GO:0001503	ossification	2.32E-06	8.92E-05
GO:0071675	regulation of mononuclear cell migration	2.35E-06	8.97E-05
GO:0031399	regulation of protein modification process	2.48E-06	9.40E-05

Table S2. Overrepresented GO terms for autosomal down-regulated genes ( $\leq 1/2$ -fold with significance)

GOID	Term	p-value	Benjamini
GO:0034660	ncRNA metabolic process	8.29E-21	2.04E-17
GO:0034470	ncRNA processing	1.55E-16	1.37E-13
GO:0022613	ribonucleoprotein complex biogenesis	4.71E-15	3.83E-12
GO:0042254	ribosome biogenesis	4.73E-14	2.91E-11
GO:0006396	RNA processing	2.31E-12	1.13E-09
GO:0006364	rRNA processing	9.71E-12	3.98E-09
GO:0016072	rRNA metabolic process	1.66E-11	5.84E-09
GO:0006520	cellular amino acid metabolic process	1.13E-09	3.48E-07
GO:0006399	tRNA metabolic process	5.60E-09	1.53E-06
GO:1901566	organonitrogen compound biosynthetic process	7.32E-08	1.80E-05
GO:1901605	alpha-amino acid metabolic process	2.13E-07	4.77E-05

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