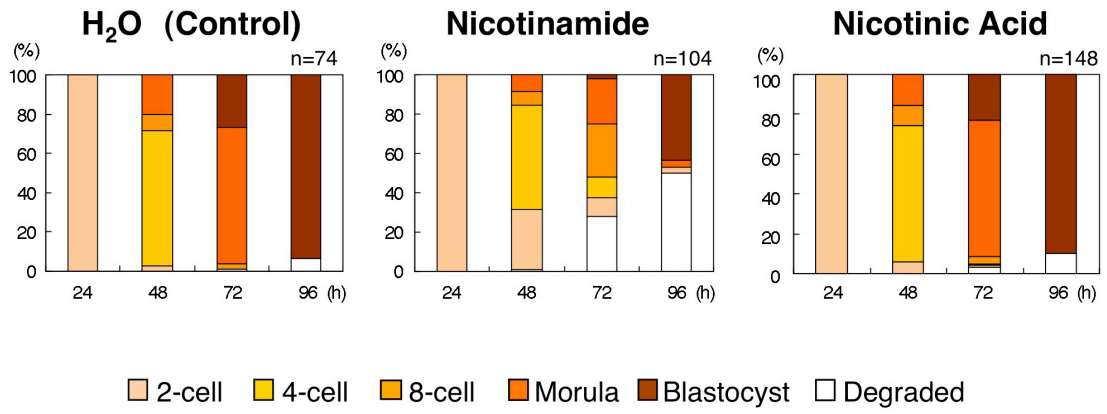

Figure S1

Effects of nicotinamide and nicotinic acid on development of in vitro fertilized and cultured embryos. Each column shows a breakdown of developmental stages at the indicated time after fertilization. Nicotinamide, but not nicotinic acid, caused developmental delay or arrest from the second cleavage.

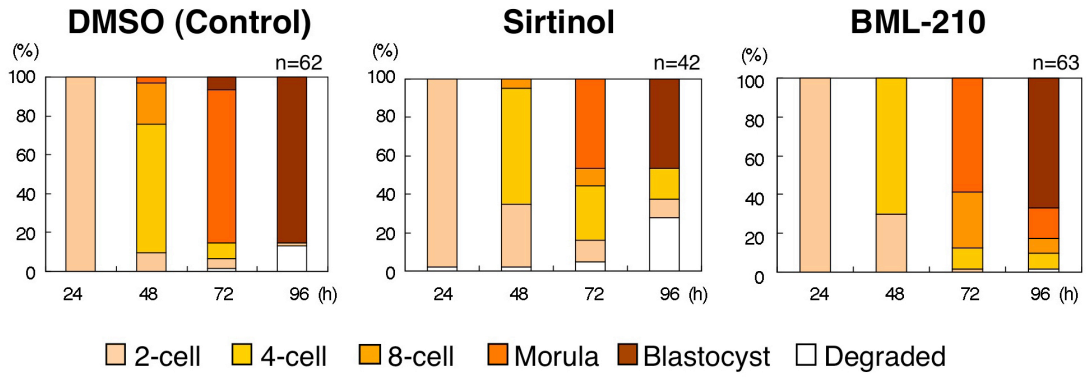

Figure S2

Effects of sirtinol and BML-210 on development of in vitro fertilized and cultured embryos. Each column shows a breakdown of developmental stages at the indicated time after fertilization. Both sirtuin inhibitors caused developmental delay or arrest from the second cleavage.

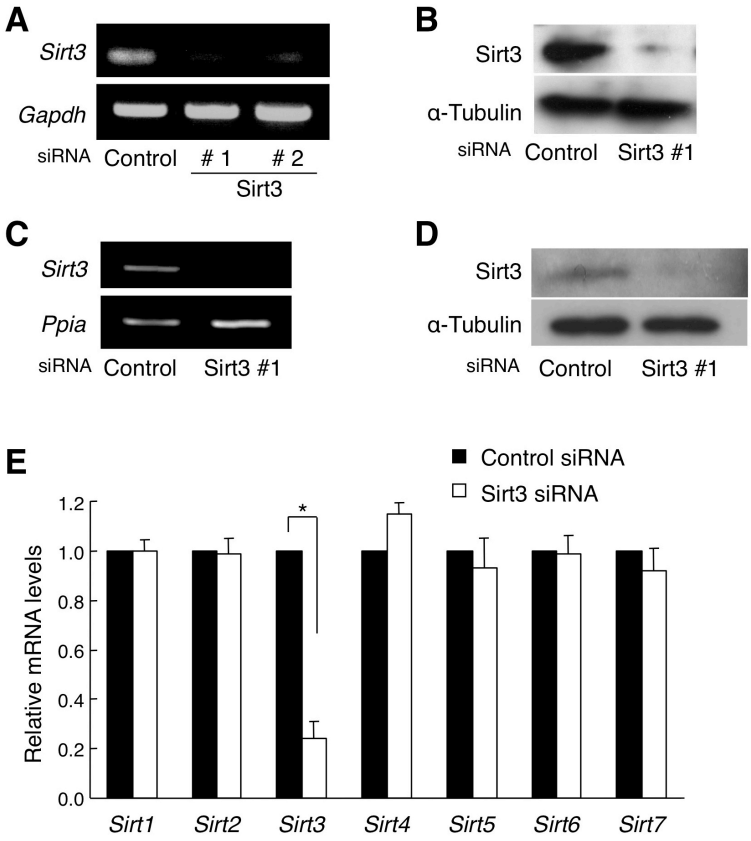

Figure S3

RNAi-mediated Sirt3 knockdown. Stealth RNAs targeting two different regions of Sirt3 transcript (#1 and #2 for nucleotides 355 to 379 and 386 to 410, respectively) downregulated Sirt3 mRNA and protein levels. (**A**) NIH-3T3 cells were transfected with Sirt3-targeted or control siRNAs. 48 hours after transfection, the effects of siRNAs were evaluated by RT-PCR analysis for Sirt3 mRNA expression. (**B**) Western blotting analysis for Sirt3 protein in NIH 3T3 cells. The decrease of Sirt3 protein by siRNA#1 transfection was detected. Blotting for α-tubulin served as an internal control. (**C**) Pronuclear stage embryos were injected with Sirt3 or control siRNAs. The effects of siRNAs were evaluated at 8-cell stage by RT-PCR analysis for Sirt3 mRNA expression. . (**D**) Western blotting analysis for Sirt3 protein in embryos. The decrease of Sirt3 protein by siRNA#1 transfection was detected at 8-cell stage. Blotting for α-tubulin served as an internal control. (**E**) Effects of Sirt3 knockdown on sirtuin gene expression. Real-time RT-PCR analysis was performed 24 h after siRNA injection. Sirt3 siRNA did not affect sirtuin gene expression except for *Sirt3* itself. Data are derived from 3 independent experiments. Statistical assessments were performed by applying Mann-Whitney U test. *$p<0.05$.

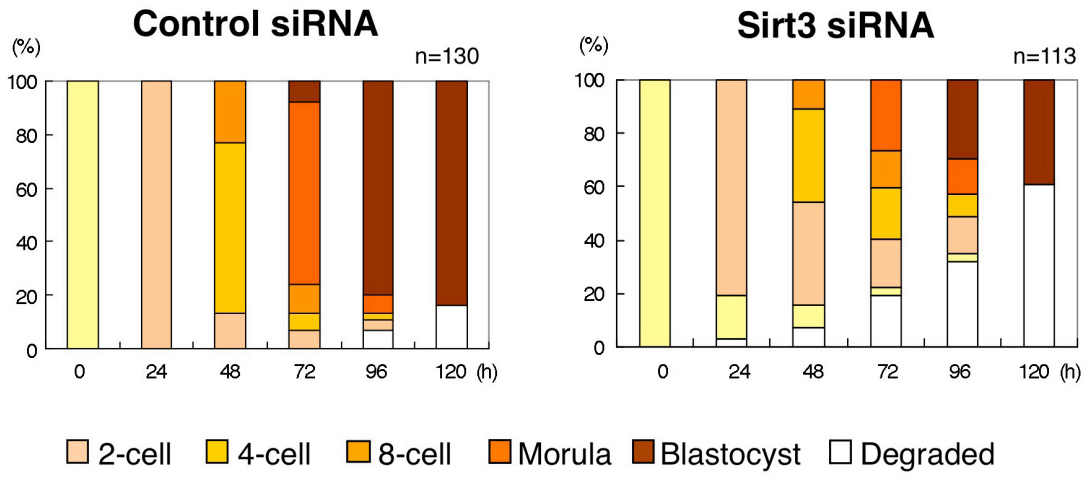

Figure S4

Effects of Sirt3 siRNA injection on development of in vitro fertilized and cultured embryos. Each column shows a breakdown of developmental stages at the indicated time after fertilization. siRNA-mediated Sirt3 knockdown caused developmental delay or arrest from the second cleavage.

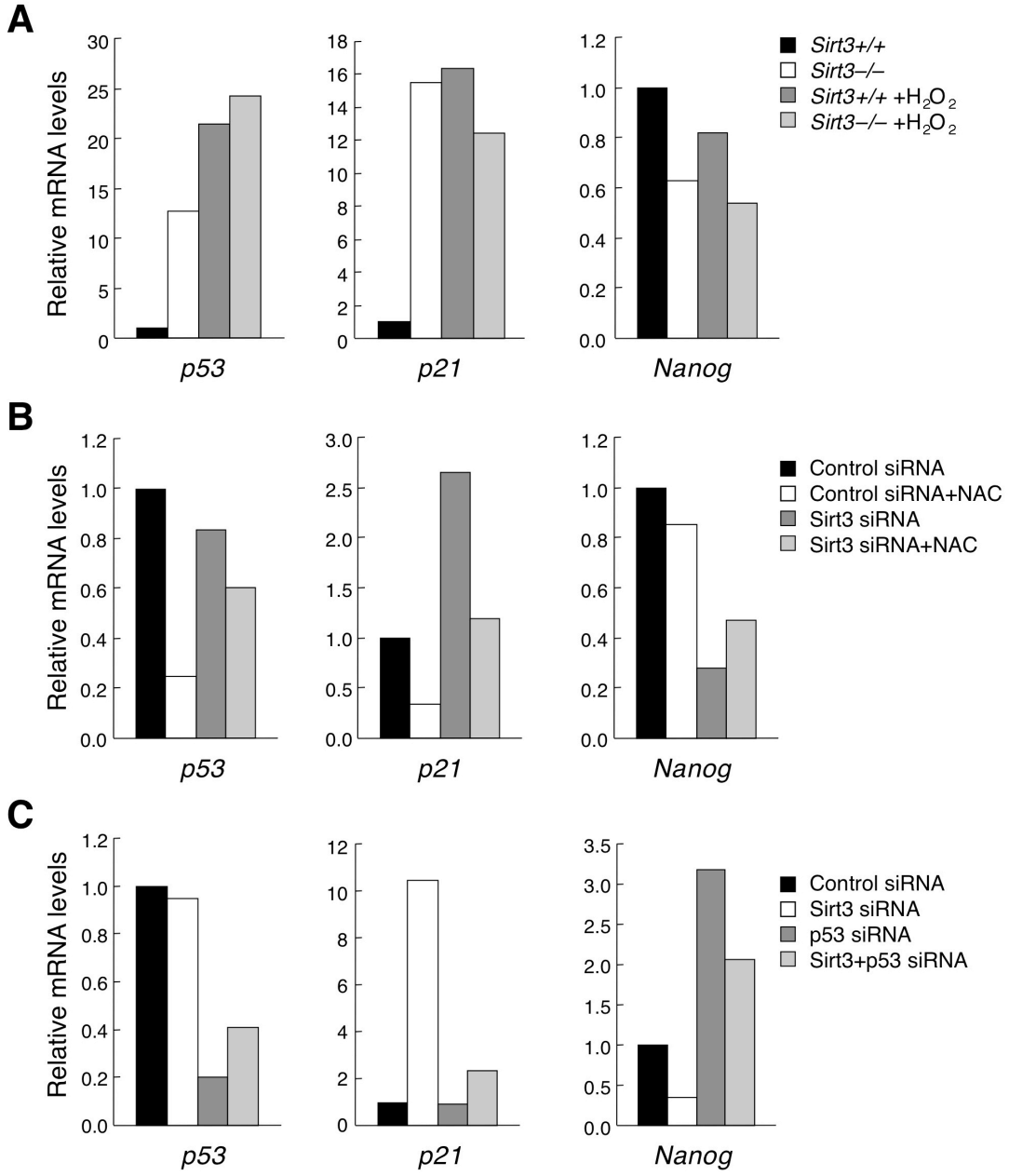

Figure S5

Quantification of *p53*, *p21* and *Nanog* mRNA levels in Sirt3-deficient preimplantation embryos in various conditions. (**A**) Effects of treatment with H_2O_2 on *p53*, *p21* and *Nanog* expression in wild-type and *Sirt3*–/– embryos. (**B**) Effects of Sirt3 knockdown and treatment with NAC *p53*, *p21* and *Nanog* expression. (**C**) Effects of p53 knockdown on Sirt3 siRNA-induced changes in *p21* and *Nanog* expression. Data are means of 2 to 4 independent experiments, examining more than 20 embryos for each experiment.

Supplemental Table S1
Primers and reaction conditions for conventional RT–PCR.

Gene	Primers		Product size (bp)	Annealing (˚C)
Sirt1	forward	5'-CCTTGGAGACTGCGATGTTA-3'	158	58
	reverse	5'-GTGTTGGTGGCAACTCTGAT-3'		
Sirt2	forward	5'-GCAGTGTCAGAGCGTGGTAA-3'	170	60
	reverse	5'-CTAGTGGTGCCTTGCTGATG-3'		
Sirt3	forward	5'-TACAGGCCCAATGTCACTCA-3'	168	63
	reverse	5'-ACAGACCGTGCATGTAGCTG-3'		
Sirt4	forward	5'-CGCTGCTCAAGATCCCTAAG-3'	179	60
	reverse	5'-GCGACACAGCTACTCCATCA-3'		
Sirt5	forward	5'-GACTCAAGACGCCAGAATCC-3'	179	60
	reverse	5'-CAGAGGATGTTCCCACCACT-3'		
Sirt6	forward	5'-CTGGTCTGGAACTCACTGCT-3'	238	60
	reverse	5'-CGGGTGTGATTGGTAGAGAG-3'		
Sirt7	forward	5'-GGCACTTGGTTGTCTACACG-3'	160	60
	reverse	5'-GTGATGCTCATGTGGGTGAG-3'		
p53	forward	5'-GACCGCCGTACAGAAGAAGA-3'	159	63
	reverse	5'-GCGGATCTTGAGGGTGAAATA-3'		
p21	forward	5'-GTACTTCCTCTGCCCTGCT-3'	171	60
	reverse	5'-TGCGCTTGGAGTGATAGA-3'		
Nanog	forward	5'-AGGGTCTGCTACTGAGATGCT-3'	364	60
	reverse	5'-CAACACCTGGTTTTTCTGCCACCG-3'		
Ppia	forward	5'-CGCGTCTCCTTCGAGCTGTTTG-3'	150	64
	reverse	5'-TGTAAAGTCACCACCCTGGCACAT-3'		
Gapdh	forward	5'-GGTGTGAACCACGAGAAATAT-3'	334	61
	reverse	5'-AGATCCACGACGGACACATT-3'		

Supplemental Table S2
Primers and reaction conditions for real-time RT-PCR.

Gene	Primers		Product size (bp)	Annealing (˚C)
Sirt1	forward	5'-CCTTGGAGACTGCGATGTTA-3'	158	63
	reverse	5'-GTGTTGGTGGCAACTCTGAT-3'		
Sirt2	forward	5'-GCAGTGTCAGAGCGTGGTAA-3'	170	63
	reverse	5'-CTAGTGGTGCCTTGCTGATG-3'		
Sirt3	forward	5'-CTGACTTCGCTTTGGCAGAT-3'	206	63
	reverse	5'-GTCCACCAGCCTTTCCACAC-3'		
Sirt4	forward	5'-CGCTGCTCAAGATCCCTAAG-3'	179	63
	reverse	5'-GCGACACAGCTACTCCATCA-3'		
Sirt5	forward	5'-AGCCAGAGACTCAAGACGCCA-3'	151	63
	reverse	5'-AGGGCGAGCTCTCTGTCCACC-3'		
Sirt6	forward	5'-TCGGGCCTGTAGAGGGGAGC-3'	174	63
	reverse	5'-CGGCGCTTAGTGGCAAGGGG-3'		
Sirt7	forward	5'-GGCACTTGGTTGTCTACACG-3'	121	60
	reverse	5'-AGGTCGGCAGCACTCACAGG-3'		
p53	forward	5'-GACCGCCGTACAGAAGAAGA-3'	159	63
	reverse	5'-GCGGATCTTGAGGGTGAAATA-3'		
p21	forward	5'-GTACTTCCTCTGCCCTGCT-3'	171	60
	reverse	5'-TGCGCTTGGAGTGATAGA-3'		
Nanog	forward	5'-GGAAGCAGAAGATGCGGACT-3'	177	60
	reverse	5'-ACCGCTTGCACTTCATCCTT-3'		
Ppia	forward	5'-CAGGTCCTGGCATCTTGTCC-3'	239	63
	reverse	5'-ATGCCCGCAAGTCAAAAGAA-3'		