Wang et al., http://www.jcb.org/cgi/content/full/jcb.200807176/DC1

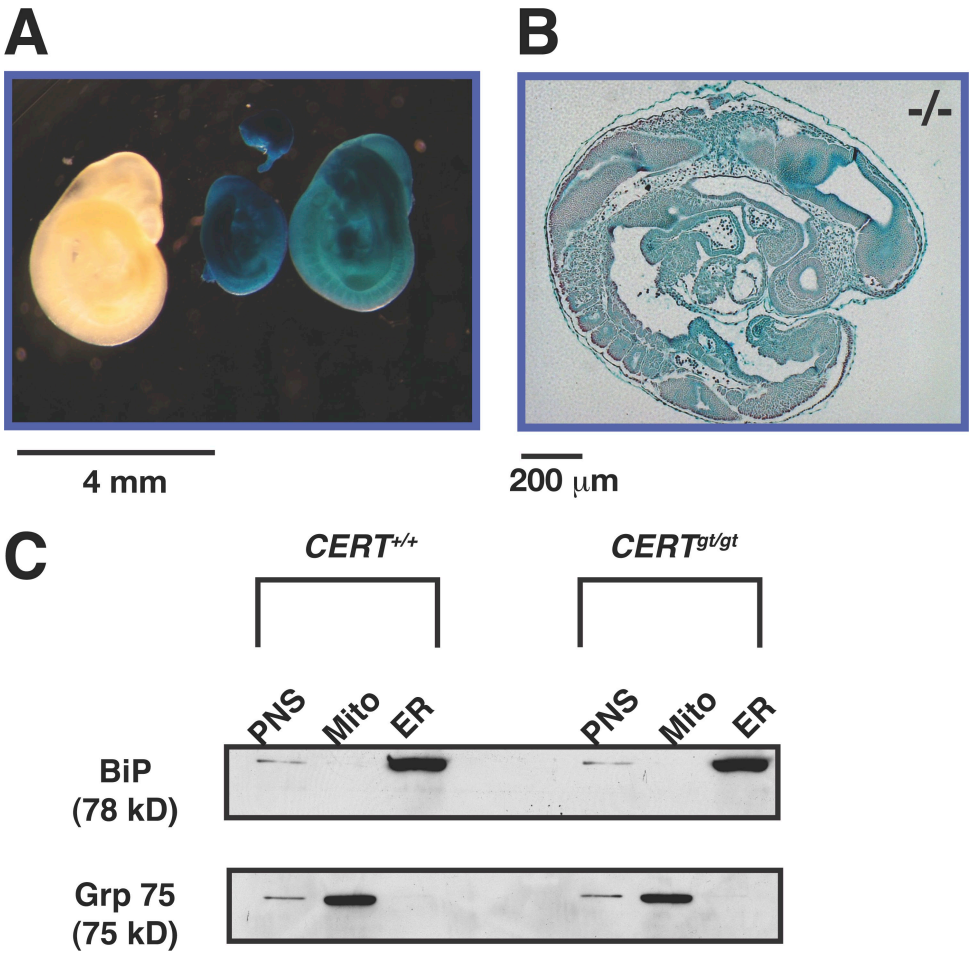


Figure S1. **The insertion of the pGT2Lxf vector results in the generation of a fusion transcript of *Cert* exons upstream of the insertion site with a reporter β-galactosidase.** (A) Efficient trapping of *Cert* by the vector with heterozygous embryos shows half the staining of β-galactosidase seen in a homozygous mutant embryo. (B) A section of E9.5 $Cert^{gt/gt}$ (−/−) embryo stained for β-galactosidase that mirrors the ubiquitous expression of *Cert*. (C) Western blots of postnuclear supernatants (PNS), mitochondrial fractions (mito), and ER fractions for the ER resident protein BiP and the mitochondrial marker protein Grp 75. There is almost no contamination of ER by Grp 75 or of mitochondria by BiP as seen in these blots. Longer exposure of the blots showed <5% of Grp 75 in ER and <1% of BiP in mitochondrial fractions.

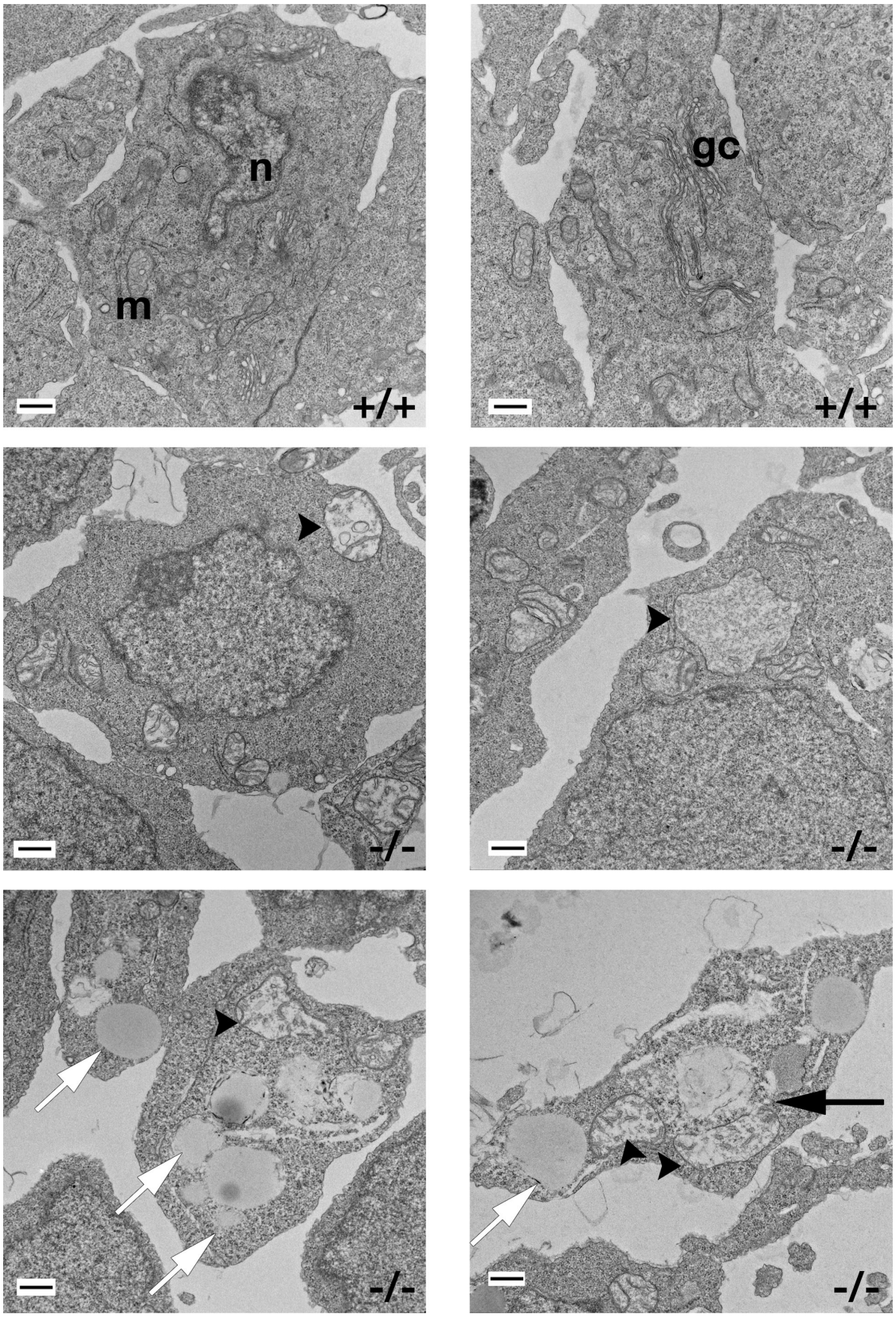


Figure S2. **Cells from the optic cup region of the developing E10.5 *Cert*$^{gt/gt}$ embryos show ER distension and mitochondrial degeneration.** The top panels show electron micrographs of cells of the developing optic cup of the wild-type embryo (+/+). n, nucleus; m, mitochondria; gc, Golgi complex. The middle and bottom panels are electron micrographs of the mutant cells (−/−) from the region. Arrowheads show swollen mitochondria in the vicinity of ER. In the bottom panels, we observe what appear to be mitochondria that have degenerated into lipid-laden structures (black arrow). The white arrows show these lipoid structures. Bars, 500 nm.

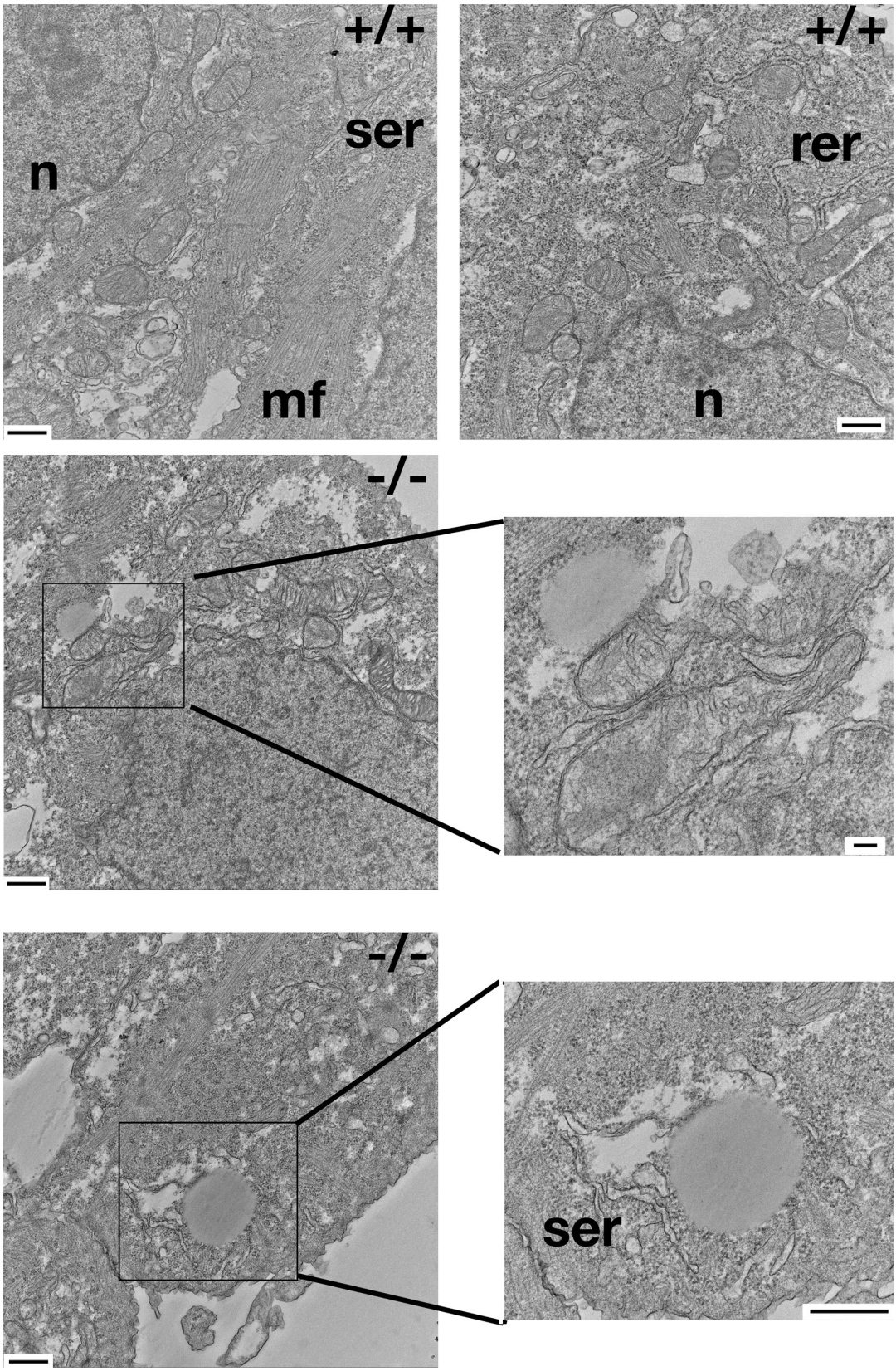


Figure S3. **Myocardial cells of *Cert*$^{gt/gt}$ E9.5 embryos show mitochondrial degeneration and accumulate lipoid material.** The top panels show the wild-type myocardial cells (n, nucleus; ser, smooth ER; rer, rough ER; mf, muscle fibers; bars, 500 nm). The middle panel shows a *Cert*$^{gt/gt}$ myocardial cell with swollen and degenerating mitochondria (bar, 500 nm). The panel to the right is an enlarged version of the mitochondria and the lipoid body in its vicinity (bar, 100 nm). The bottom panel shows swollen ER and the lipoid body in its vicinity (bar, 500 nm). The panel to the right shows an enlarged view of the indicated area (bar, 500 nm).

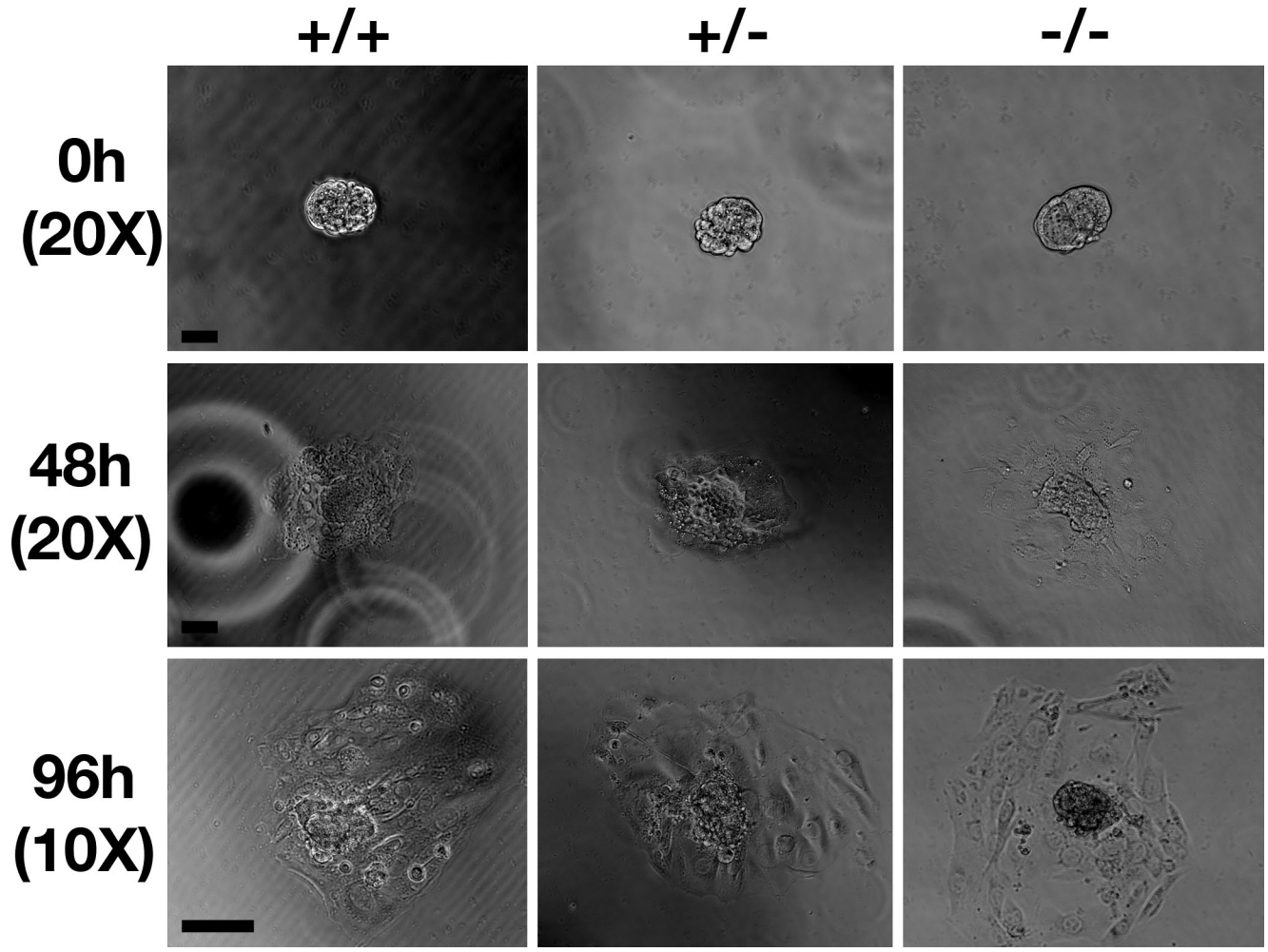


Figure S4. **The defect in *Cert*$^{gt/gt}$ embryos is not seen in preimplantation stages.** Micrographs of embryos harvested at E3.5 and cultured for 4 d. Blastocysts differentiate into inner cell mass and trophoblasts in culture. The images of cultures of E3.5 wild type (+/+), heterozygous (+/−), and *Cert*$^{gt/gt}$ (−/−) embryos were taken after 0, 48, and 96 h of culturing in the medium. The top and middle panels were observed under 20×, and the bottom panels were observed under 10× objectives. The blastocysts were genotyped by PCR (not depicted). Bars, 40 µm.

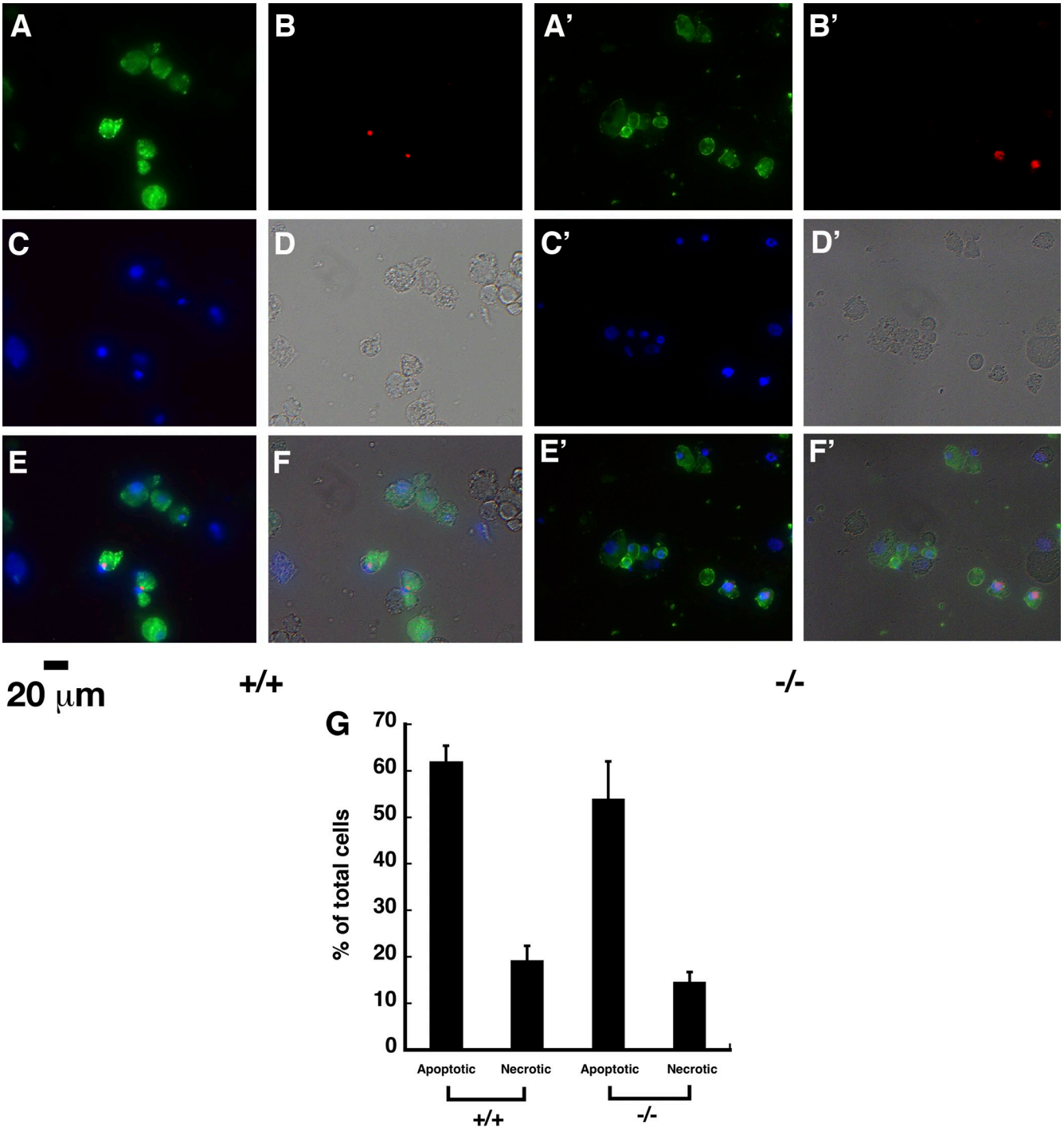
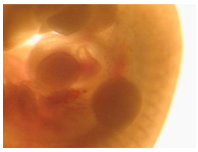


Figure S5. ***Cert*$^{gt/gt}$-derived primary MEFs are capable of undergoing apoptosis.** Primary MEFs were derived from E9.5 *Cert*$^{+/+}$ (+/+) and *Cert*$^{gt/gt}$ (−/−) embryos, treated with actinomycin D, and apoptosis was measured as described in Materials and methods. (A and B) Apoptotic cells are annexin positive (A and A′; green) and propidium iodide (B and B′; red) negative. Necrotic cells are annexin and propidium iodide positive. (C and D) The cells were also imaged using 4′ DAPI (they will stain all nuclei in the focal plane of the lens blue; C and C′) and bright field (D and D′) to count all of the cells in the field. (E and E′) Merged images of annexin, propidium iodide, and DAPI. (F and F′) Merged images of annexin, propidium iodide, and DAPI overlaid on the bright field image. Because the cells are not fixed or immobilized, some of the channels are slightly displaced during imaging. (G) Apoptosis and necrosis were measured in MEFs derived from three separate wild-type and mutant embryos and plotted as a percentage of total cells counted (n = 3; error bars indicate SD).

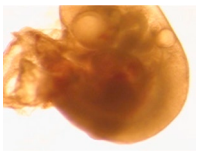
Table S1. **Tabulation of total d-18 sphingomyelins, ceramides, and hexosylceramides in preparations of whole cell extracts and d-18 sphingomyelins and ceramides in the plasma membrane preparations of *Cert*$^{+/+}$ (wild type) and *Cert*$^{gt/gt}$ (mutant) E10.5 embryos**

Analyte/ISTD	Analyte transition	Amount in 1 mg of protein											
		Total wild type			Total mutant			Wild-type plasma membrane			Mutant plasma membrane		
		Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
		pmol	*pmol*	%	*pmol*	*pmol*	%	*pmol*	*pmol*	%	*pmol*	*pmol*	%
Sphingomyelins													
d18:1$^{\Delta4}$ c12:0 ISTD	647.522→183.899	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
d18:1$^{\Delta4}$ c16:0	703.585→183.805	940.07	57.14	6.08	371.08	26.31	7.09	962.95	68.73	7.14	333.21	15.51	4.65
d18:0 c16:0	705.950→183.880	61.63	4.30	6.98	ND	ND	ND	52.56	4.65	8.85	8.11	0.81	10.00
d18:0 c18:1	731.617→183.970	60.75	3.34	5.50	7.40	0.85	11.43	125.99	13.57	10.77	28.38	2.79	9.82
d18:1$^{\Delta4}$ c18:0	731.617→183.970	126.67	9.62	7.59	65.04	2.40	3.69	147.44	11.11	7.54	33.65	0.92	2.74
d18:0 c18:0	733.600→183.880	8.00	0.41	5.17	9.95	1.53	15.38	15.55	1.43	9.18	ND	ND	ND
d18:1$^{\Delta4}$ c20:0	759.370→183.880	40.00	4.56	11.40	29.75	2.34	7.86	61.24	2.09	3.41	13.55	0.56	4.13
d18:0 c20:0	761.598→183.880	ND	ND	ND	ND	ND	ND	15.13	0.36	2.39	ND	ND	ND
d18:1$^{\Delta4}$ c22:0	787.665→183.815	213.65	8.78	4.11	88.03	5.74	6.52	370.56	20.42	5.51	73.55	6.07	8.26
d18:0 c22:0	789.980→183.880	5.20	0.39	7.54	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c23:0	801.681→183.849	48.06	4.56	9.49	26.47	2.01	7.58	94.26	0.23	0.24	22.31	2.01	8.99
d18:1$^{\Delta4}$ c24:1	813.682→183.841	442.83	17.95	4.05	245.19	18.02	7.35	466.58	48.86	10.47	218.68	15.74	7.20
d18:1$^{\Delta4}$ c24:0 or d18:0 c24:1	815.650→183.880	465.56	4.92	1.06	184.57	17.46	9.46	513.36	70.61	13.75	199.65	6.73	3.37
d18:0 c24:0	817.600→183.880	38.21	3.75	9.82	13.60	1.83	13.48	39.28	4.54	11.57	9.86	0.67	6.82
d18:1$^{\Delta4}$ c25:0	827.699→183.815	7.21	1.14	15.82	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c26:1	841.700→183.880	14.66	0.84	5.72	2.73	0.17	6.40	32.41	0.80	2.48	9.29	0.69	7.40
d18:1$^{\Delta4}$ c26:0 or d18:0 c26:1	843.698→183.880	17.47	1.56	8.91	ND	ND	ND	29.99	3.40	11.35	8.72	0.75	8.60
Ceramides													
d18:1$^{\Delta4}$ c12:0 ISTD	482.477→264.238	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
d18:1$^{\Delta4}$ c16:0	538.680→264.155	128.55	7.69	5.98	140.12	11.57	8.26	159.76	15.95	9.98	83.32	2.32	2.79
d18:0 c16:0	540.680→266.230	10.96	1.72	15.66	5.91	0.32	5.41	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c18:1	564.548→264.125	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:0 c18:0	568.690→264.155	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c18:0	566.553→266.105	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c20:0	594.576→264.155	3.57	0.02	0.61	7.22	0.59	8.22	4.76	0.20	4.26	4.38	0.27	6.17
d18:0$^{\Delta4}$ c20:0	596.730→266.230	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c22:0	622.760→264.155	33.06	4.45	13.46	37.75	3.36	8.89	42.14	4.07	9.67	18.89	1.68	8.88
d18:0 c22:0	624.760→266.230	3.76	0.37	9.98	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c24:1	648.830→264.155	113.32	11.81	10.43	129.04	4.85	3.76	116.41	7.56	6.49	122.54	20.26	16.53
d18:1 c24:0	650.652→264.126	101.55	5.95	5.86	93.65	4.57	4.88	68.56	4.64	6.76	56.16	3.65	6.49
d18:0 c24:0	652.884→266.230	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:1$^{\Delta4}$ c26:1	676.877→264.155	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.58	0.43	12.12
d18:0 c26:1	678.879→266.230	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
d18:0 c26:0	680.994→266.129	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Monohexosylceramides													
d18:1$^{\Delta4}$ c12:0 ISTD	644.499→264.109	NA	NA	NA	NA	NA	NA						
d18:1$^{\Delta4}$ c16:0	700.540→264.107	101.64	7.81	7.68	58.75	4.68	7.96						
d18:0 c16:0	702.550→266.142	ND	ND	ND	ND	ND	ND						
d18:1$^{\Delta4}$ c18:0	728.550→264.100	22.44	1.59	7.09	6.19	0.94	15.16						
d18:0 c18:0	730.550→266.120	ND	ND	ND	ND	ND	ND						
d18:1$^{\Delta4}$ c20:0	756.550→264.107	15.38	2.76	17.92	ND	ND	ND						
d18:0 c20:0	758.550→266.198	ND	ND	ND	ND	ND	ND						
d18:1$^{\Delta4}$ c22:0	784.610→264.100	54.09	1.52	2.82	33.17	3.43	10.34						
d18:0 c22:0	786.610→266.199	ND	ND	ND	ND	ND	ND						
d18:1$^{\Delta4}$ c24:1	810.650→264.107	33.95	3.16	9.31	35.43	3.04	8.59						
d18:0 c24:1	812.680→266.154	62.11	5.59	9.00	55.88	9.17	16.41						
d18:1$^{\Delta4}$ c24:0	812.680→264.114	ND	ND	ND	ND	ND	ND						
d18:0 c24:0	814.650→266.140	ND	ND	ND	ND	ND	ND						
d18:1$^{\Delta4}$ c26:1	838.650→264.100	ND	ND	ND	ND	ND	ND						
d18:0 c26:1	840.650→266.143	ND	ND	ND	ND	ND	ND						
d18:1$^{\Delta4}$ c26:0	840.650→264.107	ND	ND	ND	ND	ND	ND						
d18:0 c26:0	842.650→266.143	ND	ND	ND	ND	ND	ND						

CV, covariance; ISTD, internal standard; NA, not applicable. The totals for each category are listed as follows: total wild type, sphingomyelins: mean, 2,489.97 pmol; SD, 54.64 pmol; CV, 2.20%. Total mutant, sphingomyelins: mean, 1,043.82 pmol; SD, 45.38 pmol; CV, 4.35%. Total wild-type plasma membrane, sphingomyelins: mean, 2,927.30 pmol; SD, 13.68 pmol; CV, 0.46%. Total mutant plasma membrane, sphingomyelins: mean, 958.96 pmol; SD, 19.43; CV, 2.04%. Total wild type, ceramides: mean, 394.77 pmol; SD, 10.89 pmol; CV, 2.77%. Total mutant, ceramides: mean, 413.69 pmol; SD, 8.47 pmol; CV, 2.05%. Total wild-type plasma membrane, ceramides: mean, 391.64 pmol; SD, 84.77 pmol; CV, 8.23%. Total mutant plasma membrane, ceramides: mean, 288.87 pmol; SD, 14.82 pmol; CV, 5.13%. Total wild type, monohexosylceramides: mean, 289.61 pmol; SD, 41.58 pmol; CV, 15.61%. Total mutant, monohexosylceramides: mean, 189.42 pmol; SD, 5.66 pmol; CV, 2.99%.



Video 1. ***Cert$^{gt/gt}$* embryos have cardiovascular insufficiency.** The video depicts the cardiac contractions of a *Cert*$^{+/+}$ (wild type) E10.5 embryo. The well-enclosed heart shows rhythmic contraction, and careful examination shows the unidirectional flow of blood out of the outflow tract. The well-formed musculature and cardiac chambers make it difficult to observe the flow of blood through the entire cardiac vasculature. Also, note the slower heart beats of both embryos because of temperature-induced bradycardia. The caesarean was performed on ice, and subsequently the embryos were warmed to room temperature.



Video 2. ***Cert$^{gt/gt}$* embryos have cardiovascular insufficiency.** The video shows the cardiac contraction of an E10.5 *Cert*$^{gt/gt}$ (mutant) embryo. The contractions are rhythmic; however, there is complete regurgitation of blood during diastole back into the chambers of the heart. Also, the thin walls of the cardiac chambers can be appreciated in the video because all chambers are visible and the flow of blood can be seen through the walls in all chambers of the developing heart. Also, note the slower heart beats of both embryos because of temperature-induced bradycardia. The caesarean was performed on ice, and subsequently the embryos were warmed to room temperature.