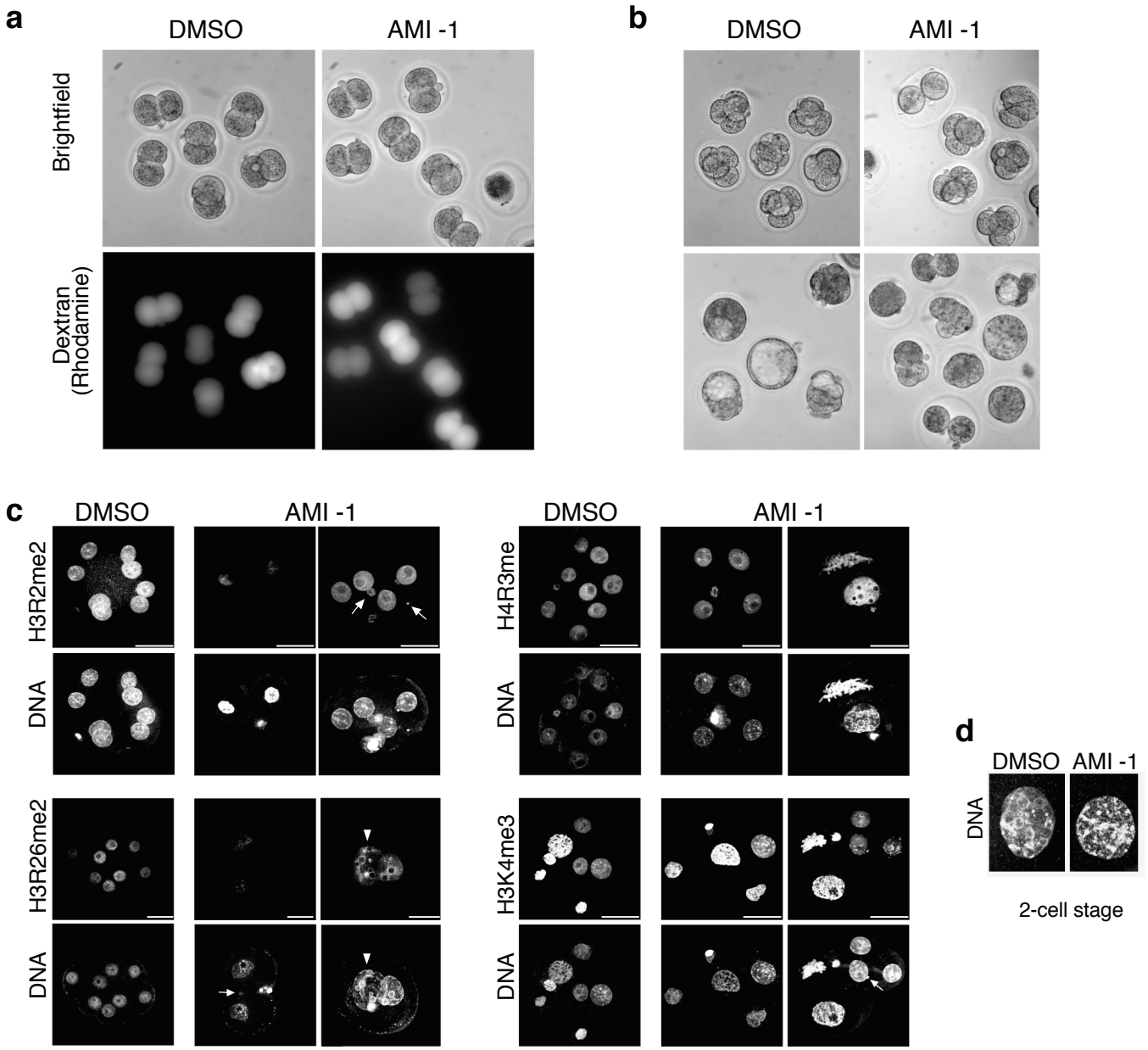

Figure S8.
Inhibition of arginine methyltransferase activity results in altered chromatin structure and impaired embryonic development.
Zygotes were microinjected with 100 μm AMI-1 or the vehicle (DMSO), together with Rhodamine-coupled Dextran as marker for injection. Note that AMI-1 shows some degree of cell permeability, albeit reduced (M. Bedford, pers.comm.). AMI-1 is a specific inhibitor of arginine methylation. In vitro, it inhibits CARM1 and to a lesser extent, PRMT1 (Cheng, D et al, 2004). In vivo, treatment of AMI-1 results in reduced levels of transcriptional activation that can be overcome by increasing amounts of PRMT1 and CARM1.
(a) Most embryos injected with AMI-1 proceeded to the 2-cell stage normally (71% n=43), although 19% either stopped their development or died at the zygote stage. All of the embryos injected only with DMSO reached to the 2-cell stage.
(b) AMI-1 treatment results in impaired development. Only 30% of AMI-1 injected embryos developped to the blastocyst stage, most of them arrested after the 1st or the 2nd cleavage (37% at the 2-cell stage, 8% at the 3- to 4-cell stage and 6% at the 8-16-cell stage). In contrast, most control (DMSO) embryos reached the blastocyst stage (80% n=38). Representative embryos after 3 and 4 days of culture are shown.

(c) Reduced levels of histone arginine methylation upon AMI-1 treatment. Control and AMI-1 treated embryos were processed for immunostaining with the indicated antibodies. Most affected residues upon AMI-1 treatment were R2 and R26 of histone H3, which were largely reduced. Levels of H4R3me were not drastically affected and levels of H3K4me3 appeared unchanged. Note the differences in DNA staining between control and AMI-1 treated embryos and the unusual larger size of the pronucleus in the zygote (white arrowheads). AMI-1 treatment led to lagging chromatin in 26% of the embryos (white arrows). Both control (DMSO) and AMI-1-treated embryos were fixed at the same time. Samples for each antibody were processed in parallel. Shown are projections of representative embryos (1 for DMSO, 2 for AMI-1) of at least 6 embryos analysed per antibody and per grouop. Sections were taken every 1 μm. Scale bar is 30 μm.
(d) Inhibition of arginine methyltransferase activity results in altered nuclear organisation in the embryos. DNA staining of one nucleus of representative control (DMSO) and AMI-1 treated 2-cell stage embryos.