1. Support for Biological Plausibility of KERs	
MIE => KE1: Histone deacetylase inhibition leads to histone acetylation increase	Biological Plausibility of the MIE => KE1 is high. Rationale: Upon the inhibition of HDAC by HDIs, the acetylation of lysine in histone remains and it leads to transcriptional activation or repression, changes in DNA replication and DNA damage repair. Activity of histone acetyltransferase (HAT) in testis nuclear protein was increased with MAA addition [Wade et al., 2008].
KE1 => KE2: Histone acetylation, increase leads to cell cycle, disrupted	Biological Plausibility of the KE1 => KE2 is moderate. Rationale: Gene transcription is regulated by histone acetylation [Struhl, 1998]. Acetylation of histones neutralizes the positive charge of the histones. Thus, less compacted DNA can be bound more easily by transcription factors and transcribed. In the models proposed for the relationship between histone acetylation and transcription, histone acetylation can be untargeted and occur at both promoter and nonpromoter regions, targeted generally to promoter regions, or targeted to specific promoters by gene-specific activator proteins [Richon et al., 2000; Struhl, 1998].
KE2 => KE3: Cell cycle, disrupted leads to apoptosis	Biological Plausibility of the KE2 => KE3 is moderate. Rationale: Prolonged cell cycle arrest will lead to either senescence or apoptosis. Especially for fast dividing and still differentiating cells, such an arrest will most certainly induce apoptosis as the normal cellular program cannot be followed.
KE3 => KE4: Apoptosis leads to spermatocyte depletion	Biological Plausibility of the KE3 => KE4 is moderate. Rationale: During development and in tissue homeostasis, apoptosis is needed to control organ size. If apoptosis is induced at a higher rate, one can assume it leading to atrophy of the target organ. Especially when target organ / target cells are fast replicating, abnormal levels of apoptosis will lead to depletion.
KE4 => AO: Spermatocyte	Biological Plausibility of the KE4 => AO is moderate. Rationale: Spermatocyte depletion is one of the main

depletion leads to testicular atrophy	characteristics of testicular atrophy.
2. Support for essentiality of KEs	
KE2: Cell cycle, disrupted	Essentiality of the KE2 is moderate. Rationale for Essentiality of KEs in the AOP: HDAC1-defecient embryonic stem cells showed reduced proliferation rates, which correlates with decreased cyclin-associated kinase activities and elevated levels of the cyclin-dependent kinase inhibitor 1A, a cell cycle regulator p21 [Lagger et al., 2002]. Loss of HDAC1 leads to significantly reduced overall deacetylase activity, hyperacetylation of a subset of histones H3 and H4 [Lagger et al., 2002].
3. Empirical support for KERs	
MIE => KE1: Histone deacetylase inhibition leads to histone acetylation, increase	Empirical Support of the MIE => KE1 is high. Rationale: HDAC inhibitors increase histone acetylation in brain [Schroeder et al., 2013]. The major empirical evidence came from the incubation of cell culture cells with small molecule compounds that inhibit HDAC enzymes followed by western blots or acid urea gel analysis. The first evidence was shown by Riggs et al. who showed that incubation of HeLa cells with n-butyrate leads to an accumulation of acetylated histone proteins [Riggs et al., 1977]. Later, it was shown that *n*-butyrate specifically increases the acetylation of histone by the incorporation of radioactive [^{3}H]acetate and analysis of the histones on acid urea gels that allow the detection of acetylated histones [Cousens et al., 1979]. TSA was shown to be an HDAC inhibitor by acid urea gel analysis in 1990 [Yoshida et al., 1990] and good evidence for VPA as an HDAC inhibitor in vitro and in vivo was shown using acetyl-specific antibodies and western blot [Gottlicher et al., 2001].

KE1 => KE2: Histone acetylation, increase leads to cell cycle, disrupted	Empirical Support of the KE1 => KE2 is moderate. Rationale: Increase in histone acetylation by HDAC inhibition induces the cell cycle regulator expression and inhibits progression through the cell cycle. Histone acetylation regulates the gene transcriptional mechanism [Struhl, 1998]. Acetylation of histones promotes the RNA polymerase reaction [Allfrey et al., 1964; Pogo et al., 1966]. Since several results supported a model in which increased histone acetylation is targeted to specific gene and occurs throughout the entire genome, not just the promoter regions, histone acetylation may lead to gene transcription of the cell cycle regulator [Richon et al., 2000].
KE2 => KE3: Cell cycle, disrupted leads to apoptosis	Empirical Support of the KE2 => KE3 is moderate. Rationale: Cell cycle arrest such as G_1 arrest and G_1/S arrest are observed in apoptosis [Li et al., 2012; Dong et al., 2010]. microRNA-1 and microRNA-206 represses CCND2, while microRNA-29 represses CCND2 and induces G_1 arrest and apoptosis in rhabdomyosarcoma [Li et al., 2012].
KE3 => KE4: Apoptosis leads to spermatocyte depletion	Empirical Support of the KE3 => KE4 is high. Rationale: MicroRNA-21 regulates the spermatogonial stem cell homeostasis, in which suppression of microRNA-21 with anti-miR-21 oligonucleotides led to apoptosis of spermatogonial stem cell-enriched germ cell cultures and the decrease in the number of spermatogonial stem cells [Niu et al., 2011].
KE4 => AO: Spermatocyte depletion leads to testicular atrophy	Empirical Support of the KE4 => AO is high. Rationale: The testicular atrophy seen in 2-methoxyethanol (2-ME), or its major metabolite MAA, treated rats in vivo and in human, and rat in vitro culture was associated with spermatocyte depletion [Beattie et al., 1984].