Supporting Information

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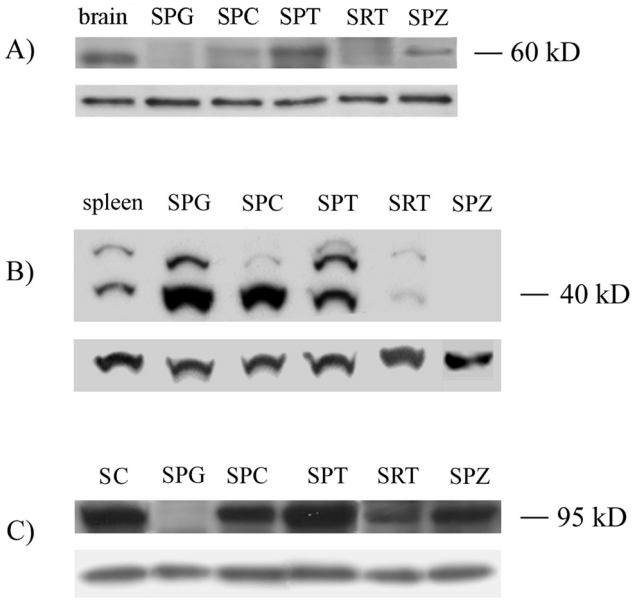

Fig. S1. Cannabinoid and TRPV1 receptor protein expression in differentiating male mouse germ cells. Immunoblot analysis of CB_1 (*A*), CB_2 (*B*), and TRPV1 receptors (*C*) was performed as described in *Methods*. Cell extracts were prepared from isolated mouse spermatogonia from 7 dpp mice or from spermatocyte and spermatids obtained by elutriation from 40 dpp mouse testes. Spermatozoa were isolated from cauda of the epididymis. Sertoli cells were obtained from 7 dpp mice. (*B*) Cell extracts from mouse brain, spleen, and spinal cord (SC) were used as positive controls of CB_1, CB_2, and TRPV1 expression, respectively. The molecular weights of the main bands were, as expected, ≈60, 40, and 95 kDa for CB_1, CB_2 and TRPV1 receptors, respectively. Actin was used as loading reference. Abbreviations are as in Fig. 1.

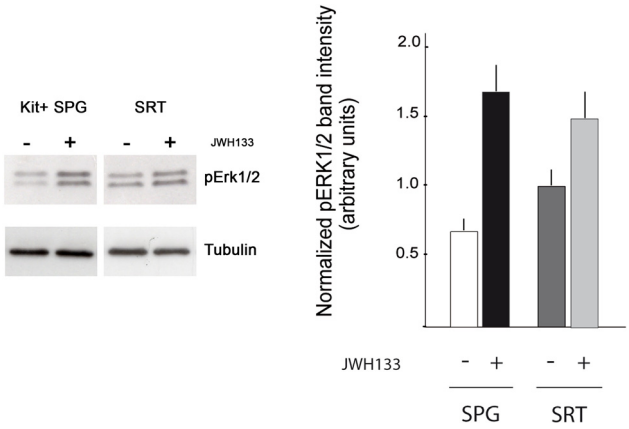

Fig. S2. MAPK Erk1/2 is activated by the CB_2 specific agonist JWH133 in purified kit-positive spermatogonia. Western blot analyses of phosphoErk1/2 in cell extracts from control or JWH133 (1 μM)-treated kit-positive spermatogonia and from control or JWH133 (1 μM)-treated Sertoli cells from 7dpp. Densitometric analysis of normalized phosphoERKs is shown on the right panel.

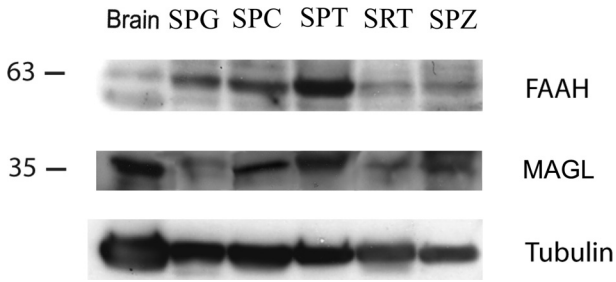

Fig. S3. FAAH and MAGL immunoblot analyses. Immunoblot analyses of FAAH (*A*) and MAGL (*B*) were performed as described in *Methods*. Cell extracts were prepared as described in *Methods* and in Fig. 2 legend. Cell extracts from mouse brain were used as a positive control. The molecular weights of the main bands were, as expected, ≈63 and 35 kDa for FAAH and MAGL, respectively. Tubulin was used as loading reference. SPG, spermatogonia; SPC, spermatocytes; SPT, spermatids; SRT, Sertoli cells; SPZ, cauda-epididymis spermatozoa

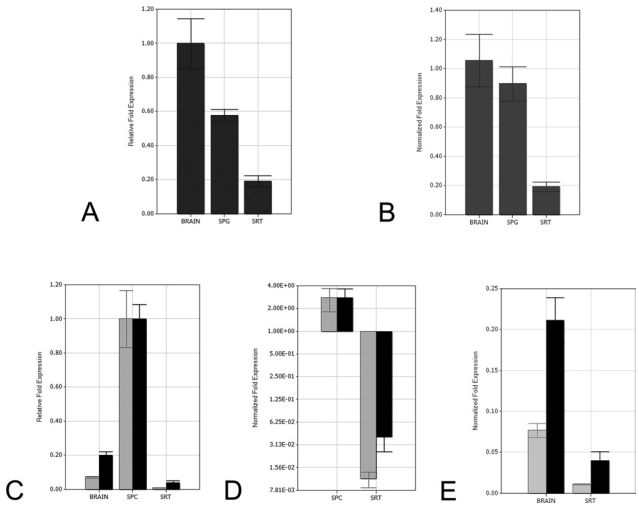

Fig. S4. Comparison of the transcriptional levels of DAGL alpha, FAAH and MAGL in germ cells, Sertoli cells and mouse brain. qRT-PCR was performed as described in *Methods* and normalized with respect to the optimal housekeeping(s). SPG, spermatogonia; SPC, spermatocytes; SRT, Sertoli cells. (*A*) DAGL alpha relative fold expression (normalized with respect to the input cDNA). (*B*) DAGL alpha normalized fold expression (using HPRT and GADPH as housekeeping genes). (*C*) MAGL (light bars) and FAAH (dark bars) relative fold expressions (normalized with respect to the input cDNA). (*D*) MAGL (light bars) and FAAH (dark bars) normalized fold expression (using HPRT and GADPH as housekeeping genes). (*E*) MAGL (light bars) and FAAH (dark bars) normalized fold expression (using beta-actin as housekeeping gene). The most highly expressed gene was considered as 1.

Table S1. Evaluation of DAGLs, FAAH, and MAGL enzymatic activities in male germ cells at different stage of differentiation and in Sertoli cells

Cell type	Tot. prot* (μg/10^6 cells)	Membr. prot† (μg/10^6 cells)	DAGLs‡		FAAH‡		MAGL§	
			Units/10^6 cells	Units/mg	Units/10^6 cells	Units/mg	Units/10^6 cells	Units/mg
SPG	63.6	10.6 ± 16.6%	10.2 ± 0.8	961 ± 78	2.99 ± 0.01	282 ± 8	28.5 ± 0.1	537 ± 12
SPC	270.6	20.6 ± 7.6%	2.1 ± 0.1	104 ± 38	5.25 ± 0.03	255 ± 1	171.0 ± 3.0	683 ± 14
SPT	56.9	7.0 ± 12.3%	ND	ND	1.75 ± 0.01	248 ± 1	41.6 ± 0.5	833 ± 9
SRT	65.5	5.1 ± 7.7%	6.0 ± 0.1	1177 ± 28	1.12 ± 0.02	220 ± 1	26.6 ± 0.3	391 ± 7

Enzymatic activities were assayed as described in *Methods* and are expressed as units/mg of protein or units/10^6 cells, taking into account the strong differences in the size and protein content exhibited by the germ cells. DAGLs, the sum of both alpha and beta isoforms in that the assay does not discriminate between the 2 enzymes. Unit is defined as the amount of enzyme that hydrolyzes 1 picomol of substrate in 1 min. SPG, spermatogonia fraction; SPC, spermatocyte fraction; SPT, spermatid fraction; SRT: Sertoli cells.

*Total protein for 10^6 cells.

†Membrane protein fraction per 10^6 cells.

‡DAGLs and FAAH enzymatic activities were assayed by utilizing the membrane protein fraction.

§MAGL enzymatic activity was assayed by utilizing the cytoplasm protein fraction.