Supplemental Figure Legends

Supplemental Figure S1. Three consecutive daily WIN 18,446 treatments of neonatal mice blocks spermatogonial differentiation. **A**) Quantitative RT-PCR analysis of markers of undifferentiated (*Zbtb16*, grey bars) and differentiating spermatogonia (*Stra8*, black bars; *Kit*, white bars). Asterisks highlight statistical significance (one asterisk, $P < 0.02$). STRA8 and ZBTB16 localization in testis cross-sections from a representative vehicle control animal (**B, D**) and animals treated with WIN 18,446 (**C**, **E**). Black arrows denote STRA8-positive spermatogonia, yellow arrows; STRA8-negative spermatogonia, and green arrows; ZBTB16-positive spermatogonia. STRA8 localization in testis cross-sections from a representative *RAREhspLacZ* vehicle control animal (**F**) and mice treated with WIN 18,446 (**G**). Black arrows denote STRA8/β-galactosidase-positive spermatogonia, and yellow arrows denote STRA8/β-galactosidase-negative spermatogonia. Analysis of STRA8- and β-galactosidase-positive cells in the vehicle control (blue bars) and WIN 18,446-treated (pink bars) animals (**H**). Y axis shows the percentage of positive cells per tubule. Asterisks highlight statistical significance (one asterisk, $P < 0.02$; two asterisks, $P < 0.001$). All scale bars denote 20 μm.

Supplemental Figure S2. ZBTB16 expression is maintained throughout the first wave of WIN 18,446/RA-synchronized spermatogenesis. ZBTB16 localization in cross-sections of vehicle control animals (**A**-**D**) and animals treated with WIN 18,446 for 7 consecutive days followed by a single injection of RA. Testes from treated animals were collected prior to injection (0 hours; **E**) or 24 hours (**F**), 4 days (**G**) and 8 days (**H**) post injection. Green arrows denote ZBTB16-positive spermatogonia. All scale bars represent 50 μm.

Supplemental Figure S3. Synchronous spermatogonial differentiation also occurs in the absence of an injection of RA after WIN 18,446 treatment of neonatal mice. STRA8 localization in cross-sections of vehicle control animals (**A**) and animals treated with WIN 18,446 for 7 consecutive days followed by a single injection of DMSO (RA vehicle). Testes from vehicle-injected animals were collected 24 (**B**) and 48 hours (**C**) post injection. Black arrows denote STRA8-positive spermatogonia, red arrows denote STRA8-positive preleptotene spermatocytes, yellow arrows denote STRA8-negative spermatogonia and the asterisks denote tubule cross-sections containing very few or no STRA8-positive cells. All scale bars represent 50 μm.

Figure S1.

BOR-Papers in Press. Published as DOI: 10.1095/biolreprod.112.105346

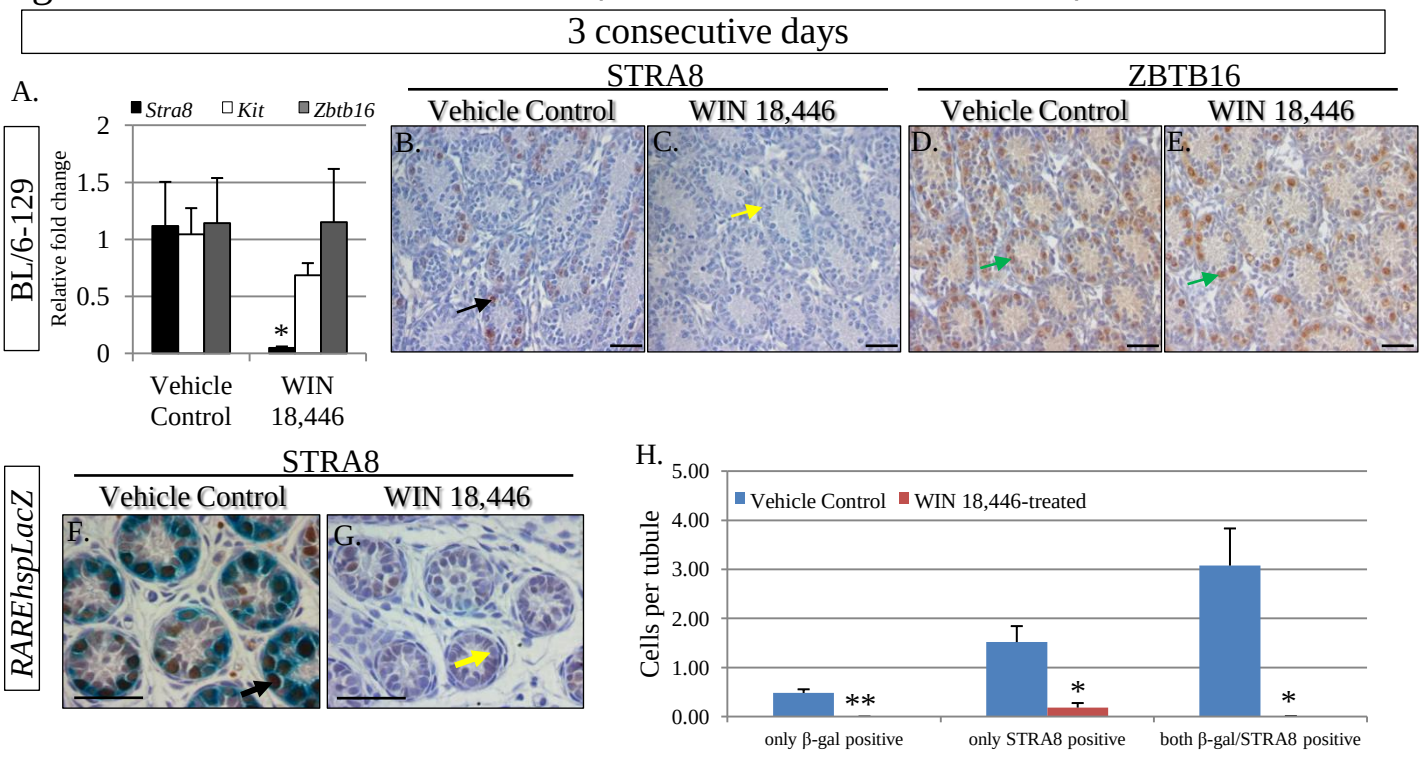

Figure S2.

BOR-Papers in Press. Published as DOI: 10.1095/biolreprod.112.105346

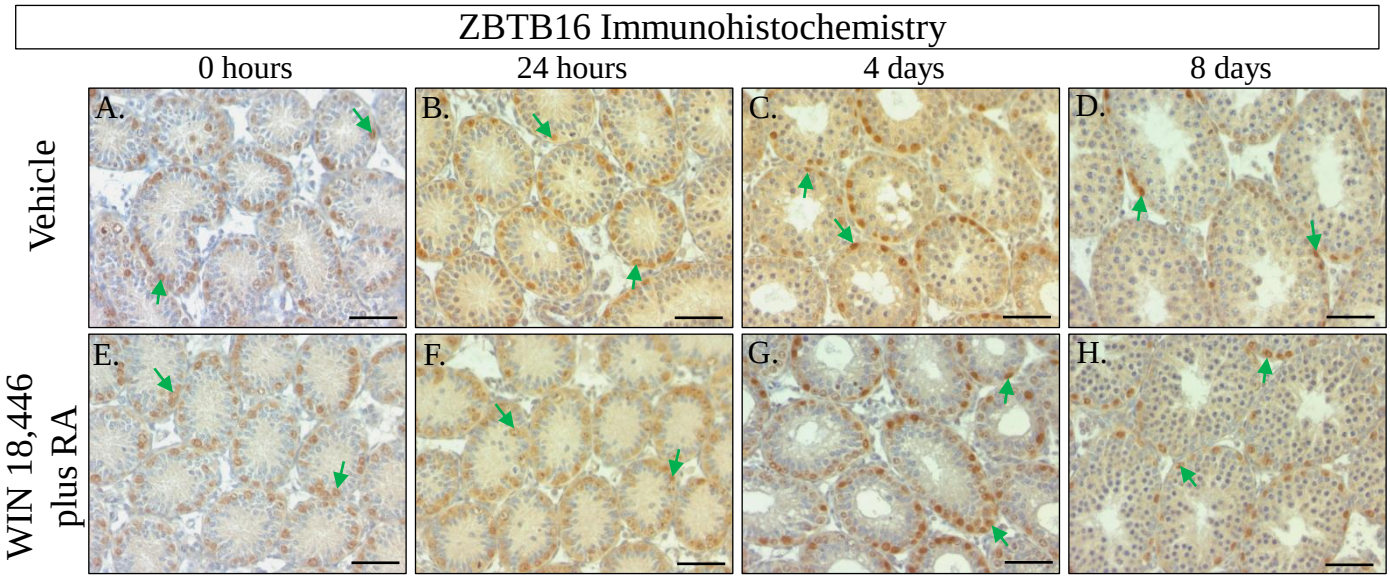

Figure S3.

BOR-Papers in Press. Published as DOI: 10.1095/biolreprod.112.105346

STRA8 immunohistochemistry

Vehicle only

WIN 18,446 + Vehicle