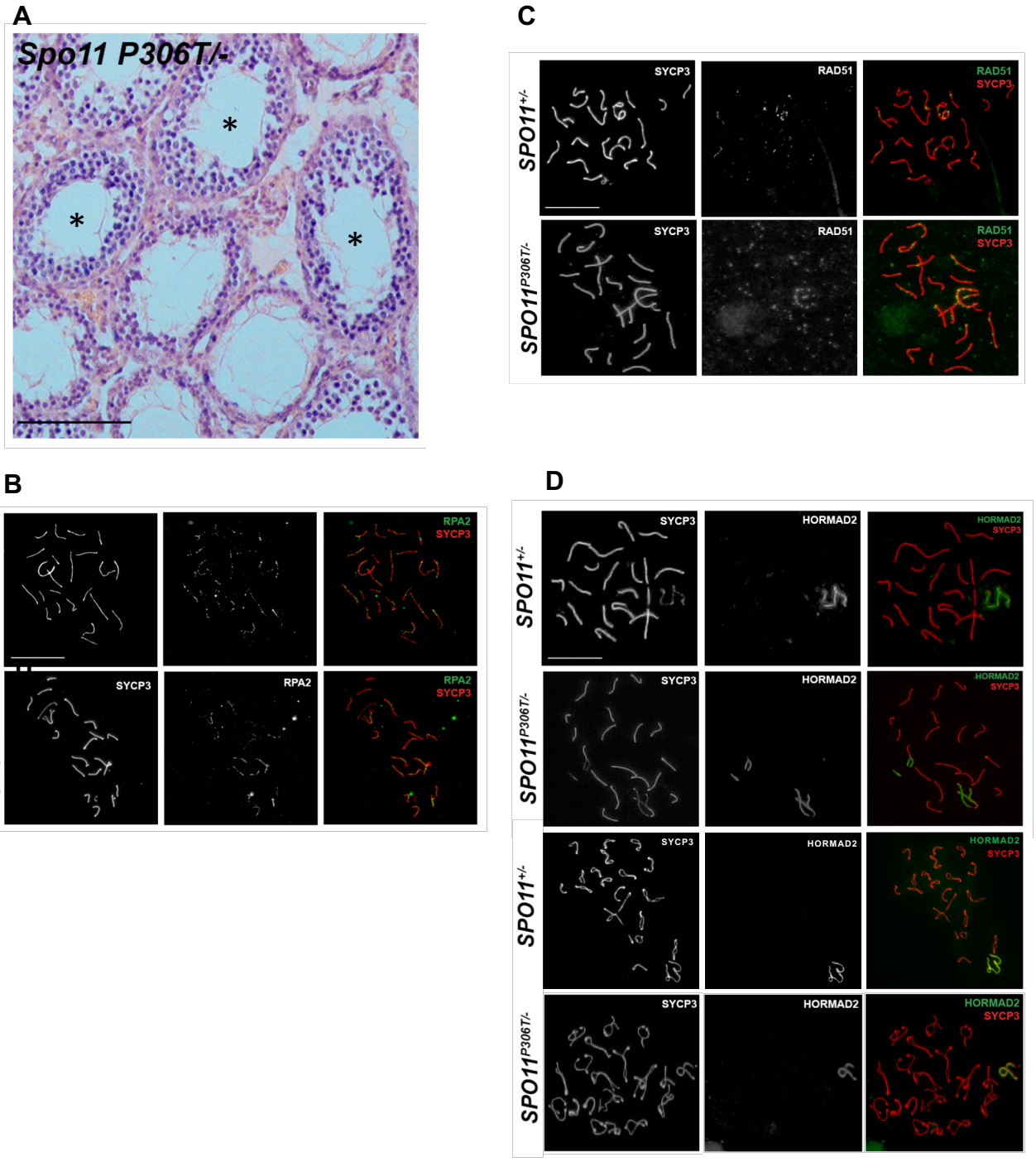
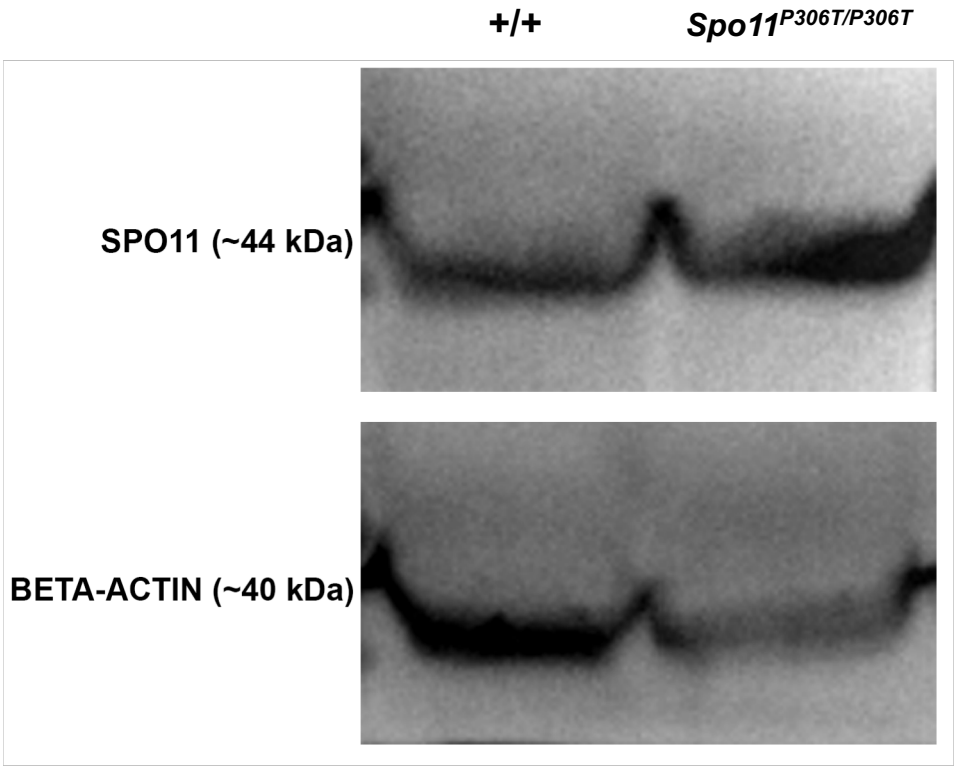


Supplementary Figure 1. *Spo11*$^{P306T/-}$ males and females are severely germ cell depleted, and spermatocytes have drastically reduced meiotic DSBs. **(A)** H&E stained testis cross-section from an eight-week old *Spo11*$^{P306T/-}$ male. Size bar = 75μm. Notice postmeiotic germ cells in all tubules. The tubules with asterisks appear to contain arrested spermatocytes. (**B, C)** Representative pachytene spermatocyte surface spread immunolabeled with RPA2 or RAD51 plus SYCP3. Quantification is plotted in Fig. 4. **(D)** Representative spermatocyte chromosome spreads immunolabeled with SYCP3 and HORMAD2 at pachynema (top two rows) and diplonema (bottom two rows). Whereas the only the XY body labels with HORMAD2, indicating asynapsed regions, there are also autosomes asynapsed at pachynema. Normal diplotene nuclei were also observed.



Supplemental Figure 2. Western blot of whole testis protein probed with anti-SPO11 antibody.

Supplementary Table 1

Purpose	Name	Sequence (5'-3')	Amplicon	RE Digest	Citation
Genotyping SPO11 P306T	Forward	GTTTCACCATGCCTGGACTT	309 bp	HaeIII WT = 247bp, 62bp P306T = 125bp, 122bp, 62bp	
	Reverse	CCTGTAGCTCAGCCAGGTTC			
Genotyping SPO11 null	Mutant Forward	CTGCAGGTTTGATGATTCTGT	200 bp	N/A	Baudat et al. 2000
	Mutant Reverse	CATCAGAAGCTGACTCTAGAG			
	WT Forward	GCAATGCTCATTCTGTGTTG	200 bp	Yes/No	
	WT Reverse	GGCACTTTCAGCATACAGGA			
Genotyping CHEK2 null	Mutant Forward	CCAAAGAAGTCTCCGTTGCT	157 bp	Yes/No	Hirao et al. 2002
	Mutant Reverse	CAAATTAAGGGCCAGCTCATTC			
	WT Forward	CCTTATGTGGTACGCCCACT	150 bp	Yes/No	
	WT Reverse	CCACCTCATCCAACCAGACT			
RT-qPCR: DDX3Y	Forward	GGGCGCTATATACCTCCTCAC	[redacted]		Royo et al. 2010
	Reverse	TCCAAAACTGCTGTAGGCATC			
RT-qPCR: EIF2S3Y	Forward	AACTATGCTGAATGGGGCAG			Royo et al. 2010
	Reverse	TAATTTCAATGGCAGCCAGG			
RT-qPCR: SRY	Forward	GAGAGCATGGAGGGCCATG			Zwingman et al. 1993
	Reverse	GAGTACAGGTGTGCAGCTC			
RT-qPCR: UBE2B	Forward	CGCCCCATCTGAAAACAACA			Royo et al. 2010
	Reverse	TGGGACTCCATCGATTCTGC			
RT-qPCR: USP9Y	Forward	ATGGCAGGTTGCACATTCAC			Royo et al. 2010
	Reverse	CAGTCCATCTTGATCATTTGG			
RT-qPCR: ZFY1	Forward	GCCAGTGCTCTCTTAAACCAA			Royo et al. 2010
	Reverse	TGAGTACACAAAGTCCCAGCA			
RT-qPCR: ZFY1/2	Forward	TGGATGAAGCATCTCCAGAA			Royo et al. 2010
	Reverse	CCACCAGCATCTTCATCTCC			
RT-qPCR: KDM6A	Forward	TACAGGCTCAGTTGTGTAACCT			Jiang et al. 2013
	Reverse	CTGCGGGAATTGGTAGGCTC			
RT-qPCR: MID1	Forward	AGAGAAACACAGAACTGGAGACT			Nakamura et al. 2017
	Reverse	CAGTTTGGCTTCTTGACGGG			
RT-qPCR:	Forward	CTCATCCTCATGTCTTCTCCG			

Supplementary Table 1

XIST	Reverse	GATTCCAGATAGACAGGCTGG	[redacted]	Kobayashi et al. 2006
RT-qPCR: GAPDH	Forward	CTTTGTCAAGCTCATTTCCTGG		Kartha et al. 2016
	Reverse	TCTTGCTCAGTGTCCTTGC		

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