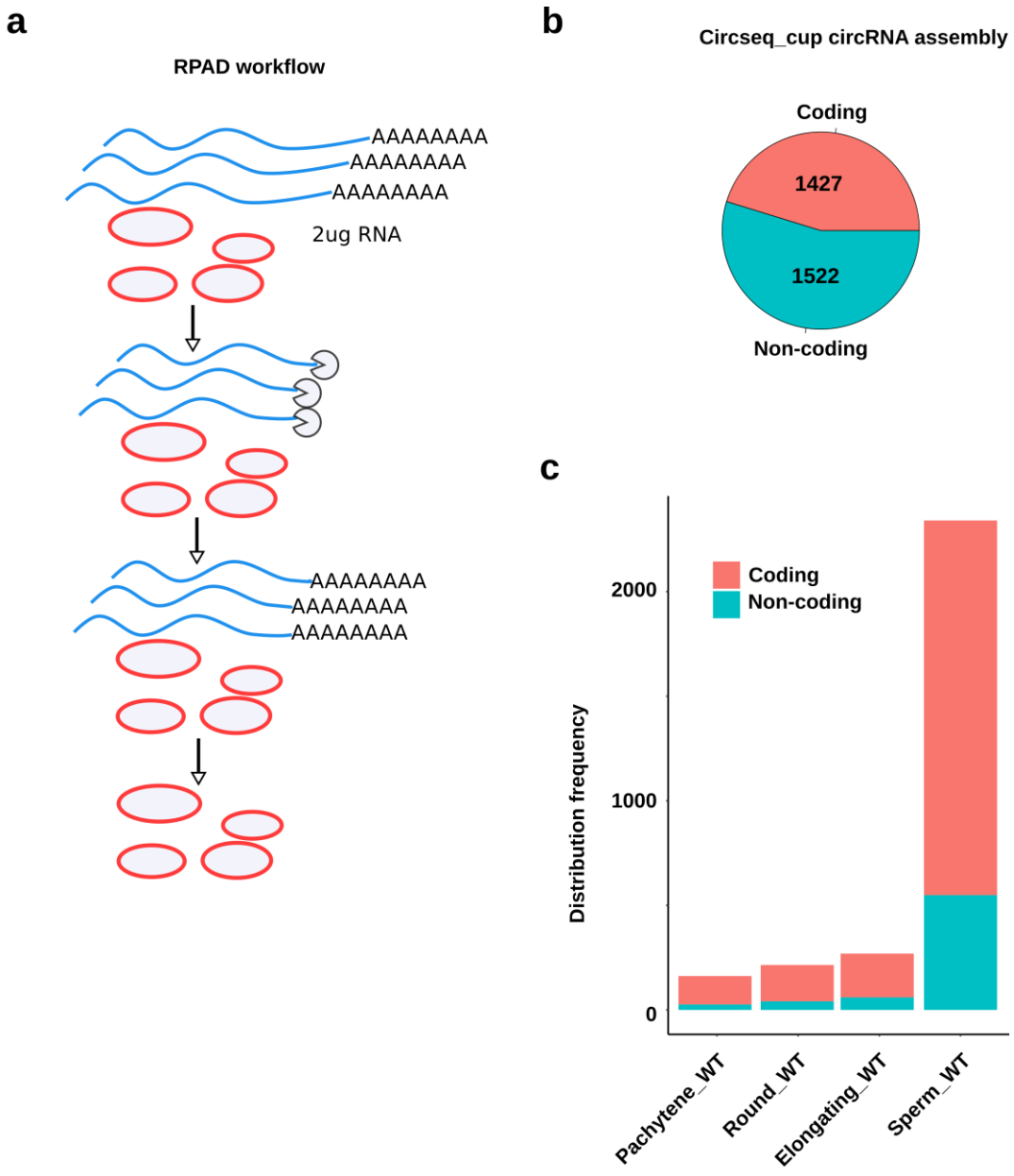
a
RPAD workflow
AAAAAAAA
AAAAAAAA
AAAAAAAA
2ug RNA
AAAAAAAA
AAAAAAAA
AAAAAAAA
b
Circseq_cup circRNA assembly
Coding
1427
1522
Non-coding
c
Coding
Non-coding
Distribution frequency
2000
1000
0
Pachytene_WT
Round_WT
Elongating_WT
Sperm_WT

Fig.S1. RPAD (RNase R treatment followed by polyadenylation and poly[A]- RNA depletion)-seq-based circRNA identification in mouse testes. **a** Schematics showing the RPAD workflow. The whole testis total RNA is first treated with RNase R to remove most of the linear RNAs. The remaining linear RNAs after RNase R treatment are then polyadenylated followed by removal of poly(A)-RNAs using immunoprecipitation. After these two steps of linear RNA removal, circular RNAs get highly enriched. **b** Distribution of full-length circRNAs with or without coding potential. Circseq_cup was used to construct the full-length sequences of circRNAs from the RPAD data. ~a half of the full-length circRNAs have coding potential. **c** Distribution of the full-length circRNAs with or without coding potential in pachytene spermatocytes, round and elongating spermatids, as well as spermatozoa purified from wild-type (WT) mice. The bar plots show intersections between the circRNAs identified in purified spermatogenic cell types by RNase R treatment-based conventional RNA-seq and the full-length coding/noncoding circRNAs identified from total testes using RPAD-seq. Data are presented as sum of two biological replicates (n=2).