



Supplementary Figure S7. PTN signalling pathways changes during human testicular aging; related to Fig. 6. (A) Information flow of significant signalling pathways received by germ cell populations in the young (red columns) and old (blue columns) groups. (B) Circle plot showing the interaction probability between all cell populations of each ligand-receptor pair in the PTN signalling pathway. Edge width represents the communication

(continued)

Supplementary Figure S7. Continued

probability. In the PTN signalling pathway, PTN acts as a ligand for the receptors, NCL, SDC2, SDC3, and SDC4. **(C)** Immunostaining and quantification of STRA8 in young men's seminiferous tubules cultured *in vitro* of young men. Left, representative image of STRA8 in seminiferous tubules of Ctrl and Anti-PTN groups. Right, quantification of the number of STRA8+ cells per seminiferous tubule. Scale bars, 35 μm . Bars represent the mean with SEM of 60 independent tubules per group (Ctrl, n = 6 samples; Anti-PTN, n = 6 samples). Significance was determined by Student's t-test. *** $P < 0.001$. **(D)** Immunostaining and quantification of STRA8 in old men's seminiferous tubules cultured *in vitro*. Left, representative image of STRA8 in seminiferous tubules of Ctrl and PTN groups. Right, quantification of the number of STRA8+ cells per seminiferous tubule. Scale bars, 35 μm . Bars represent the mean with SEM of 60 independent tubules per group (Ctrl, n = 6 samples; PTN, n = 6 samples). Significance was determined by Student's t-test. *** $P < 0.001$. Ctrl, control group; PTN, recombinant PTN protein group; Anti-PTN, anti-PTN antibody group.