Nemp1 regulates mechanotransduction responses to substrate stiffness

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Nuclear Envelope Membrane Protein 1 (Nemp1) is a highly conserved transmembrane nuclear envelope protein required for fertility in Drosophila, C. elegans, zebrafish, and mice (Tsatskis et. al, 2020). Human genome-wide association studies show an association between Nemp1 and early menopause. The mammalian ovary is composed of a mechanically stiff, highly cross-linked cortex filled with primordial follicles that constitute the ovarian reserve. *Nemp1*^{KO} female mice have a drastically reduced ovarian reserve. Loss of ovarian reserve can lead to ovarian failure that results in premature menopause. Atomic Force Microscopy (AFM) and micropipette aspiration technique revealed that NEMP protein supports the mechanical stiffness of the nucleus in cell lines and *Nemp1*^{KO} oocytes respectively (Tsatskis et. al, 2020). These data led us to hypothesize that Nemp1 has a role in mechano-transduction.

Here we show that Nemp1 is required to prevent apoptosis, promote cell proliferation, and maintain nuclear shape in tissue culture adherent cells. We hypothesized that the loss of cells upon Nemp1 depletion in culture is due to the increased mechanical stresses on the nucleus caused by culture on glass and plastic. Remarkably, we found that NEMP1$^{-/-}$ cell survival is rescued in soft substrate (10kPa) but not in stiff substrate (120kPa). Transcriptional cofactor YAP1 is known to be regulated by extracellular matrix (ECM) stiffness and stretching, with stiff environments promoting YAP nuclear localization. We find that nuclear YAP is reduced in Nemp1-depleted cells on stiff substrates. *Nemp1*^{KO} lung fibroblasts also showed no nuclear YAP upon stretching for 120 minutes compared to *WT* lung fibroblasts. Significantly, restoring nuclear YAP via expression of (YAP S→A) rescues cell loss in Nemp1-depleted cells. YAP nuclear translocation in the stiff substrate is rescued in Nemp1-depleted cells by inducing actin polymerization with jasplakinolide, indicating that Nemp1 regulation of YAP is via a mechanism dependent on F-actin polymerization. The mammalian ovary is composed of a mechanically stiff cortex filled with primordial follicles that constitute the ovarian reserve. *Nemp1*^{KO} female mice have a reduced ovarian reserve. We hypothesized that the loss of the ovarian reserve in *Nemp1*^{KO} was due to the mechanically stiff cortical ovary environment. Reducing stiffness of the ovary with LOX mutants rescues fertility. Hence, Nemp1 mechanically supports the NE of the primordial follicles in a mechanically stiff environment to support female fertility.