

# **Pml represses tumor progression through inhibition of mTOR**

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*Running title: PML and mTOR in tumor progression*

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Character count: 27774

**Supporting Information Figure 1. Pml expression in kidney.**

**A.** Immunohistochemical analysis of gelsolin expression as a marker of distal tubules and collecting ducts in kidney cortex from 18-months old mice of the indicated genotype. Scale bars: 50 $\mu$ M.

**B.** Immunohistochemical analysis of Pml expression in kidney medulla and cortex of wt 8 months old mice. Arrows indicate Pml expression in cortical tubules. Scale bars: 50 $\mu$ M.

**C.** Specific Pml nuclear staining is absent in section from *Pml*<sup>-/-</sup> from 8-months old mice. Scale bars: 50 $\mu$ M.

**D.** Immunohistochemical analysis of gelsolin, Pml/phospho-S6, and NaCl cotransporter (NCC) performed on consecutive kidney sections from 8-months old mice (scale bar: 50 $\mu$ M). Arrows point to the same kidney tubules that in consecutive sections express all three markers analyzed. All images are representative of  $\geq 3$  independent experiments showing the same result.

**Supporting Information Figure 2. Liver TORC1 activity upon compound inactivation of *Pml* and *Tsc2*.**

**A.** Western blot analysis of total S6K and 4EBP1 in liver and kidney extracts from 4-months old mice of the indicated genotypes.

**B.** Western blot analysis of phospho/total proteins in liver and kidney extracts from 4-months old mice of the indicated genotypes. Graph on the right represents ratios of phospho/total proteins.

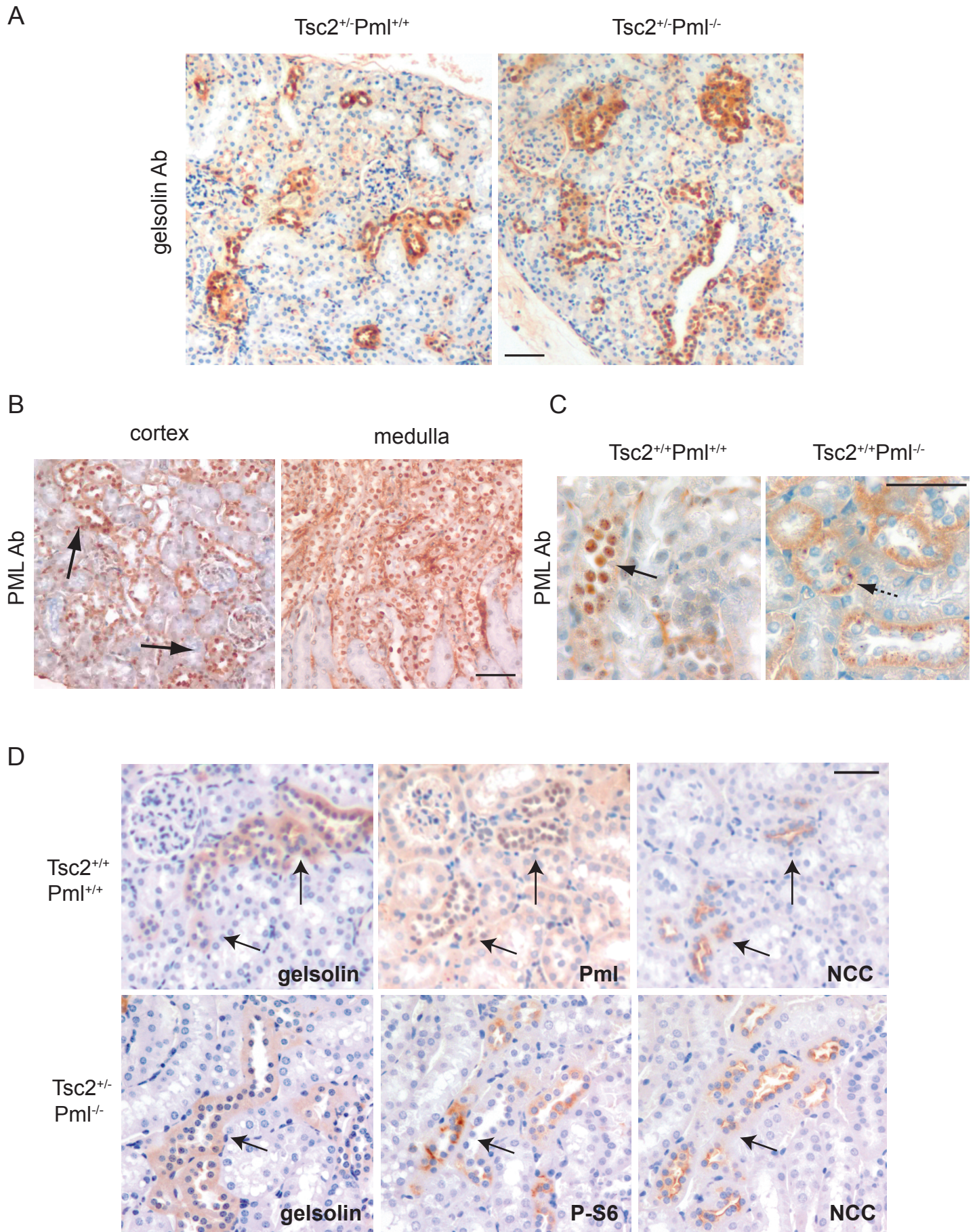
**C.** Western blot analysis of phospho/total 4EBP1 in kidney extracts from 4-months old mice of the indicated genotypes. Graph on the right represents average ratios of phospho/total 4EBP1 of the samples in Supporting Information Figure 2B and C. P value was calculated by Student's t-test.

**Supporting Information Figure 3. Compound inactivation of *Pml* and *Tsc2* does not increase Akt phosphorylation in the kidney.**

Representative immunohistochemistry of phospho-Akt (Ser473) on kidney sections from 8 months old mice of the indicated genotype (scale bar: 50 $\mu$ M). The staining appears non-specific as it accumulates at the lumen of tubuli and at the cilia.

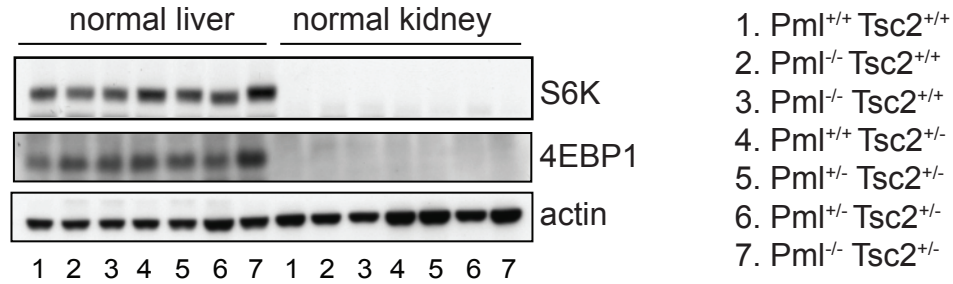
**Supporting Information Figure 4. TORC1 activation in *Pml*<sup>-/-</sup>*Tsc2*<sup>+/-</sup> tumors upon *Tsc2* LOH.**

Ratios of phospho-S6 and phospho-4EBP1 over total S6 and 4EBP1 are indicated in *Tsc2*<sup>+/-</sup> mice of different *Pml* genotypes. *Pml*<sup>-/-</sup> tumors are further divided according to the status of *Tsc2* LOH, indicating that TORC1 activity is slightly higher in tumors that have undergone *Tsc2* LOH.

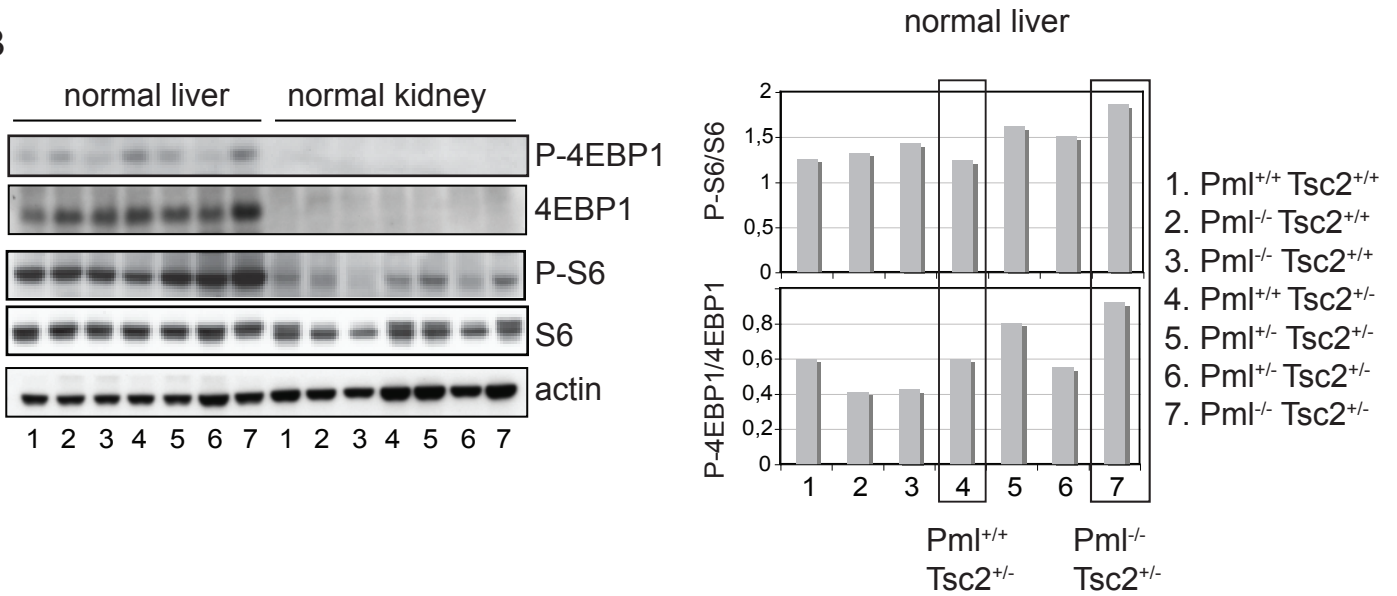




A



B



C

