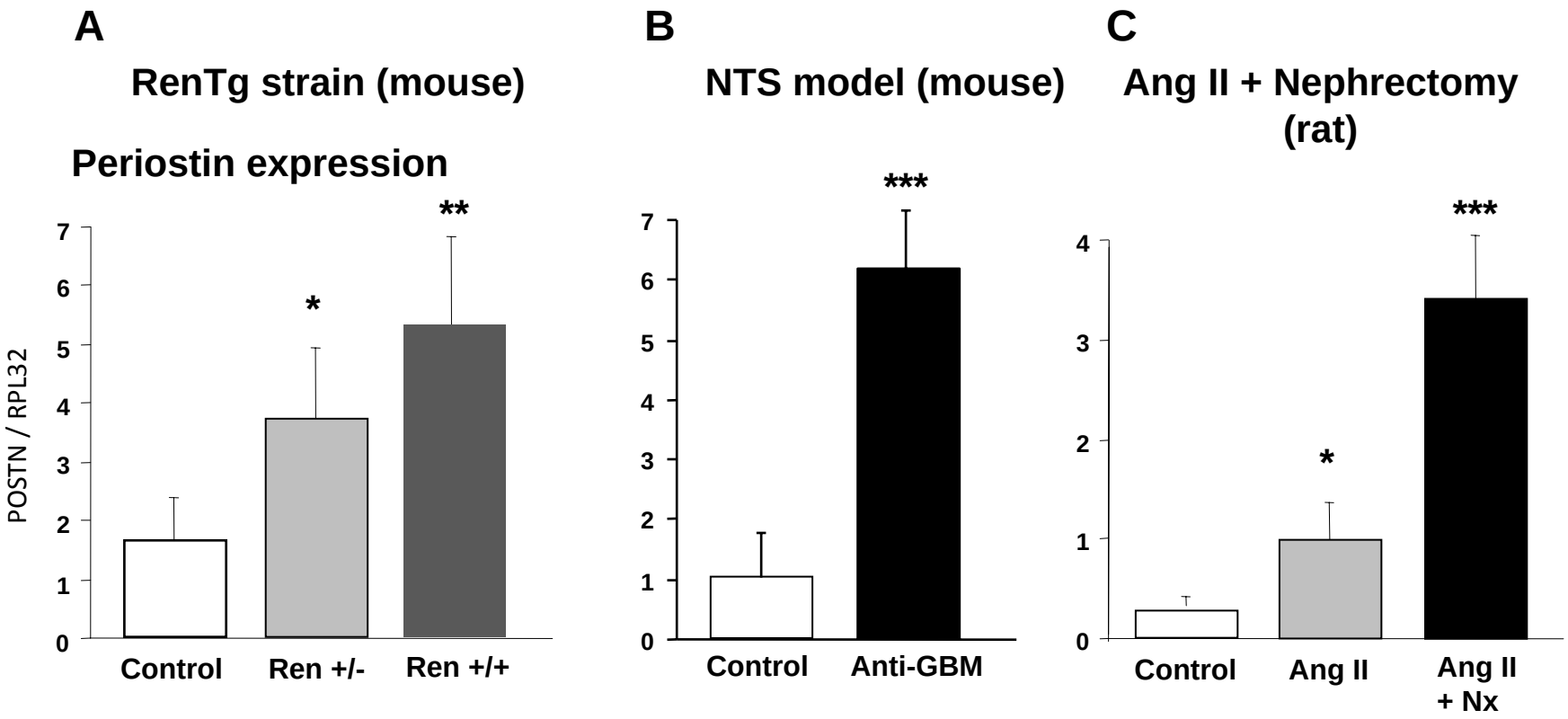

Supplemental Figure 1

Figure 1S: Periostin expression is increased with the severity or stage of progression in three different fibrotic models of renal disease: transgenic mice overexpressing renin (n=6/strain), mice injected with nephrotoxic serum (n=8/group) and rats receiving continuous infusion of angiotensin II and subjected or not to unilateral nephrectomy (n=6/group). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs control.

METHODS

Animal models:

RenTg mice. These mice express a renin transgene inserted into a liver-specific locus and driven by a liver specific promoter/enhancer. The renin coding sequence (Ren2/1d) is a synthetic cDNA consisting of parts of the Ren-2 and Ren-1d genes modified to include glycosylation sites for increased stability, a furin cleavage site to enable prorenin to active renin processing to occur in the liver and allow secretion of active renin into the blood stream. Thus, this transgenic strain expresses renin ectopically at a constant high level in the liver and leads to elevated protein levels of active renin. In previous studies we have shown that these mice develop, with age, renal disease characterized by enhanced albuminuria, peri-vascular and periglomerular inflammation, glomerular ischemia, glomerulosclerosis, mesangial expansion and tubular dilation. (Huby et al 2009, 2012, Kavvadas et al 2013). Three groups of animals were used: mice carrying one or two copies of transgene and age-matched littermate controls (n=6/group). Mice were sacrificed at the age of 5 months. Based on previous experience, the first fibrotic lesions appear in the renal cortex at this age.

Crescentic Glomerulonephritis: Anti-GBM glomerulonephritis was induced by retro-orbital injection of decomplementated nephrotoxic serum, as previously described (Kerroch et al 2012). Sixteen female 129/SV mice (Janvier, Le Genest-St-Isle, France) of 3-4 months of age and weighing between 18 and 22 g were used. NTS was injected in 8 mice during three consecutive days (total 23 μl/g BW), whereas the remaining 8 mice were considered as controls and received 0.9% NaCl solution. All mice were sacrificed at day 9 after nephrotoxic serum injections.

Ang II + Nephrectomy: Male Sprague-Dawley rats, weighing 250 g, were anesthetized and infused subcutaneously with angiotensin II (200 ng/kg/min, Sigma Chemical, MO, USA) using osmotic mini pumps (Alzet, CA, USA) with or without unilateral nephrectomy (n=6/group). Six rats receiving infusion of 0.9% NaCl were used as controls. All animals were sacrificed 4 weeks after the beginning of angiotensin II infusions.

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