A. WT

<table>
<thead>
<tr>
<th>kDa</th>
<th>Vehicle</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td></td>
<td></td>
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<tr>
<td>130</td>
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<td></td>
</tr>
<tr>
<td>36</td>
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<td></td>
</tr>
</tbody>
</table>

pNCC (T45,50,55)

NCC

β-actin

B. 

\[ \text{pNCC/NCC (Fold Change)} \]

\[ \begin{array}{c}
\text{Vehicle} \\
\text{Furosemide}
\end{array} \]

C. WT

<table>
<thead>
<tr>
<th>kDa</th>
<th>Vehicle</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>36</td>
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</tbody>
</table>

pWNK4 (S64)

WNK4

β-actin

D. 

\[ \text{WNK4/β-actin (Fold Change)} \]

\[ \begin{array}{c}
\text{Vehicle} \\
\text{Furosemide}
\end{array} \]

*
A.

<table>
<thead>
<tr>
<th>kDa</th>
<th>Vehicle</th>
<th>R-586 (0.60 µg/ml/min)</th>
</tr>
</thead>
<tbody>
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<tr>
<td>130</td>
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<tr>
<td>36</td>
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</tr>
</tbody>
</table>

- pNCC (T45,50,55)
- NCC
- pSPAK (S373)
- SPAK
- β-actin

B.

![Bar chart showing fold change in pNCC/NCC and pSPAK/SPAK expression](chart)

- **: Significant difference at p < 0.01
- *: Significant difference at p < 0.05

Vehicle vs. R-568
Increased calcium and salt delivery to the distal nephron

Hypercalcemia

Calcium excretion

Salt recovery

Increased calcium and salt delivery to the distal nephron

Hypercalcemia

Calcium excretion

Salt recovery