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**A.**

A gel image with protein bands labeled as CaSR, mWNK4 (HA), and β-actin. The gel is loaded with samples including Empty, CaSR WT, CaSR-E228K, and CaSR-R185Q. The molecular weight markers are indicated in kDa (250, 130, 36).

**B.**

A bar graph showing the fold change of WNK4/β-actin expression. The samples include WNK4 WT, CaSR WT, CaSR-E228K, and CaSR-R185Q with or without Klhl3 DNA. The graph includes error bars and statistical significance markers (ns, **, ***).

**C.**

A bar graph showing the fold change of WNK4/β-actin expression in control and transfection conditions with or without Klhl3 DNA. The samples include Control, CaSR WT, CaSR-E228K, and CaSR-R185Q.

**D.**

A bar graph showing the fold change of WNK4/β-actin expression in control and transfection conditions with or without Klhl3 DNA. The samples include Control, CaSR WT, CaSR-E228K, and CaSR-R185Q.
A. 

<table>
<thead>
<tr>
<th>kDa</th>
<th>Empty</th>
<th>CaSR wt</th>
<th>CaSR E228K</th>
<th>CaSR R185Q</th>
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<tbody>
<tr>
<td>72</td>
<td>72</td>
<td>+</td>
<td></td>
<td></td>
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</tbody>
</table>

IP: Flag

pRRXS

KLHL3(Flag)

B. 

<table>
<thead>
<tr>
<th>kDa</th>
<th>Control</th>
<th>CaSR WT</th>
<th>CaSR E228K</th>
<th>CaSR R185Q</th>
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</thead>
<tbody>
<tr>
<td>72</td>
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</table>

IP: Flag

pRRXS

KLHL3(Flag)

C. 

<table>
<thead>
<tr>
<th>kDa</th>
<th>+</th>
<th>+</th>
<th>R-568</th>
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</thead>
<tbody>
<tr>
<td>130</td>
<td>pWNK4 S1196</td>
<td>mWNK4 (HA)</td>
<td>pS1196/WNK4</td>
</tr>
<tr>
<td>95</td>
<td>pSPAK (S373)</td>
<td>hSPAK (HA)</td>
<td>CaSR</td>
</tr>
<tr>
<td>36</td>
<td>pERK</td>
<td>β-actin</td>
<td>R-568</td>
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</tbody>
</table>

D. 

<table>
<thead>
<tr>
<th>kDa</th>
<th>+</th>
<th>+</th>
<th>R-568</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>CaSR</td>
<td>mWNK4 (HA)</td>
<td>pSPAK/SPAK</td>
</tr>
<tr>
<td>130</td>
<td>hSPAK (HA)</td>
<td>pERK</td>
<td>R-568</td>
</tr>
<tr>
<td>95</td>
<td>β-actin</td>
<td>CaSR</td>
<td>mWNK4-5A</td>
</tr>
<tr>
<td>36</td>
<td>R-568</td>
<td>CaSR</td>
<td>mWNK4-5A</td>
</tr>
</tbody>
</table>

**Fold Change**
A. 

WT

Vehicle | R-568

kDa

130 -
pNKCC2 (S91)

130 -
NKCC2

B.

pNCC/NCC (Fold Change)

Vehicle | R-568

0.5 | 1.0 | 1.5 | 2.0 | 2.5

*p

C.

WT

Vehicle | R-568

kDa

130 -
pNCC (T45,50,55)

130 -
NCC

130 -
pWNK4 (S64)

130 -
WNK4

36 -
β-actin

D.

pNCC/NCC (Fold Change)

Vehicle | R-568

0.5 | 1.0 | 1.5 | 2.0 | 2.5

*p

E. 

Vehicle

NCC

DAPI

Merge

R-568

NCC

DAPI

Merge

F. 

SPAK^{243A/243A}

kDa

Vehicle | R-568

130 -
pNCC (T45,50,55)

130 -
NCC

36 -
β-actin

G. 

pNCC/NCC (Fold Change)

Vehicle | R-568

ns
**A.**

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

**B.**

Vehicle | Furosemide
---|---

pNCC/NCC (Fold Change)

**C.**

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

**D.**

Vehicle | Furosemide
---|---

WNK4/β-actin (Fold Change)
A. 

<table>
<thead>
<tr>
<th>kDa</th>
<th>Vehicle</th>
<th>R-586 (0.60 µg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

B. 

- pNCC/NCC (Fold Change)
  - Vehicle
  - R-568
- pSPAK/SPAK (Fold Change)
  - Vehicle
  - R-568

** and * indicate statistical significance.
Increased calcium and salt delivery to the distal nephron

Hypercalcemia

Calcium excretion

Salt recovery

Transcellular voltage

Transepithelial voltage