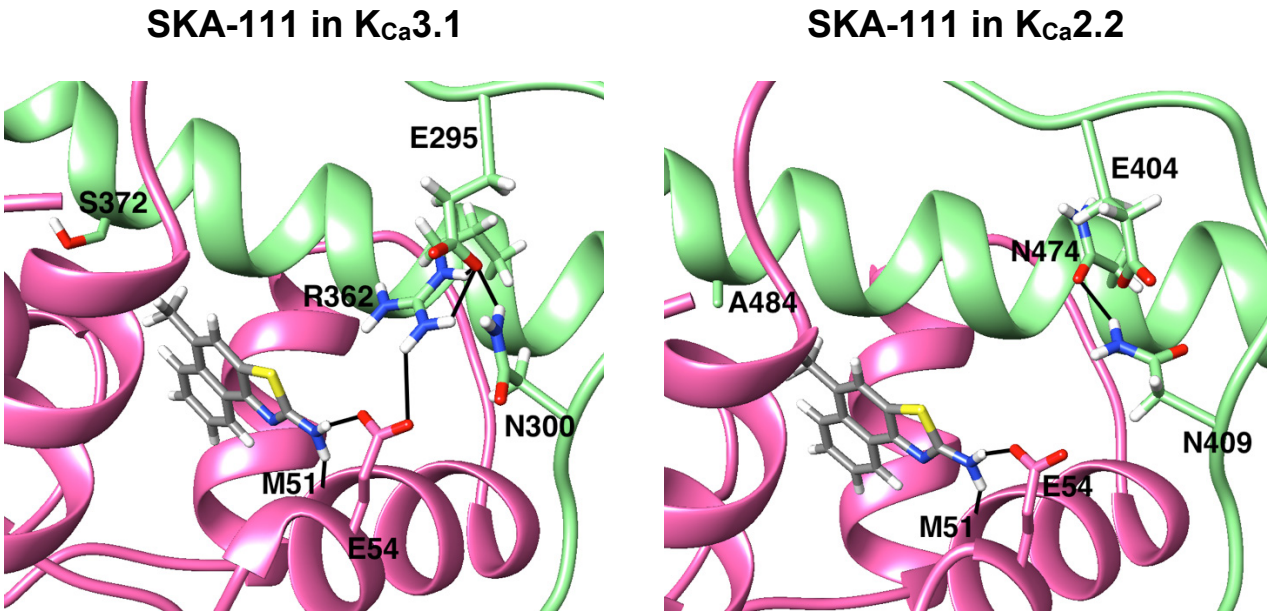
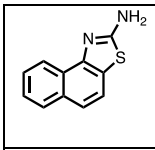
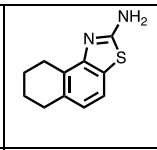
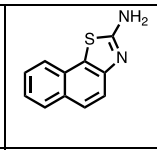
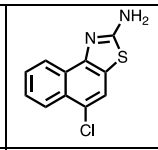
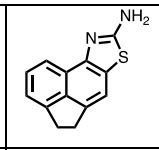
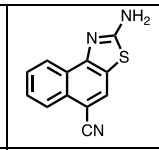
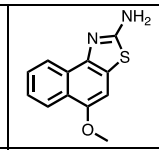
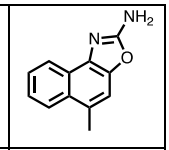
Supplementary Fig.1



Docking model of $K_{Ca}3.1$ and $K_{Ca}2.2$ CaM-BD/CaM complexes with SKA-111. In the $K_{Ca}3.1$ model the amino group of the benzothiazole ring system of SKA-111 forms hydrogen bonds with M51 and E54 in calmodulin. E54 is further stabilized by an extensive hydrogen bond network with R362, E295 and N300 in the $K_{Ca}3.1$ channel model, which we hypothesized to be responsible for the 100-fold $K_{Ca}3.1$ selectivity of SKA-111. In the $K_{Ca}2.2$ model, however, SKA-111 forms only hydrogen bonds with M51 and E54. Due to the shorter length of the N474 side chain in $K_{Ca}2.2$ E54 is not stabilized by additional hydrogen bonds. The CaM-BD is shown in light green; CaM is colored pink.

The eight 2-aminobenzothiazoles shown below, which all display some degree of selectivity for $K_{Ca}3.1$ over $K_{Ca}2$ channels, converged on similar binding poses as SKA-111 with an extensive hydrogen bond network centered around R362 in the $K_{Ca}3.1$ model and hydrogen bonds between the -NH_2 group and M51 and E54 in CaM in both the $K_{Ca}3.1$ and the $K_{Ca}2.2$ model.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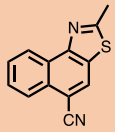
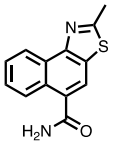
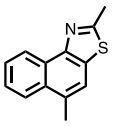
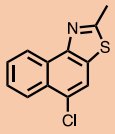
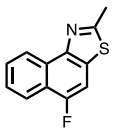
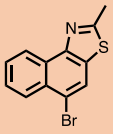
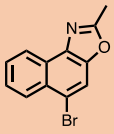
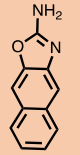
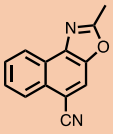
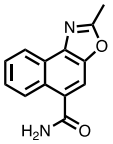
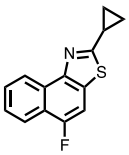
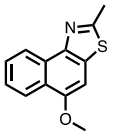
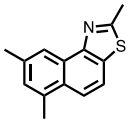
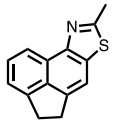
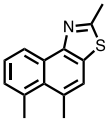
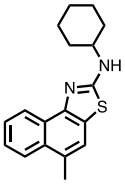
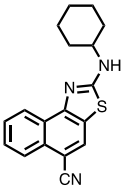
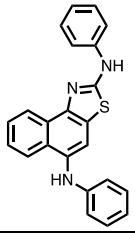
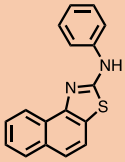
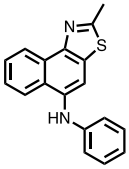
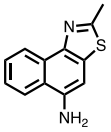
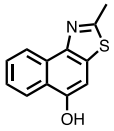
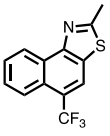
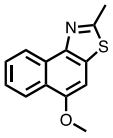
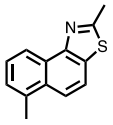
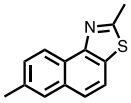
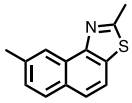
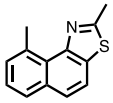
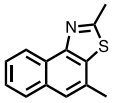
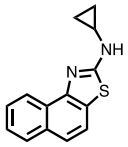
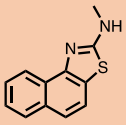
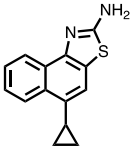
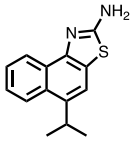
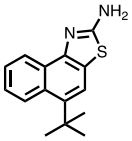
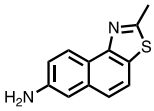
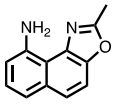
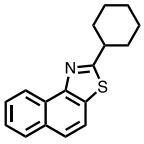
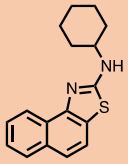
Supplementary Fig.2
Chemical structures of the 168 virtually designed and docked compounds.
Highlighted compounds were synthesized and tested.

SKA-126 	SKA-127 	SKA-128 	SKA-129 	SKA-130 	SKA-131 	SKA-132 	SKA-133 
SKA-134 	SKA-135 	SKA-136 	SKA-137 	SKA-138 	SKA-139 	SKA-140 	SKA-141 
SKA-142 	SKA-143 	SKA-144 	SKA-145 	SKA-146 	SKA-147 	SKA-148 	SKA-149 
SKA-150 	SKA-151 	SKA-152 	SKA-153 	SKA-154 	SKA-155 	SKA-156 	SKA-157 
SKA-158 	SKA-159 	SKA-160 	SKA-161 	SKA-162 	SKA-163 	SKA-164 	SKA-165 
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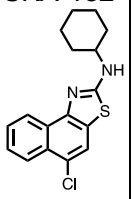
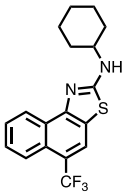
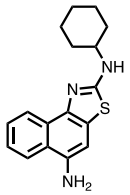
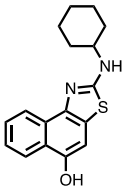
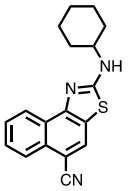
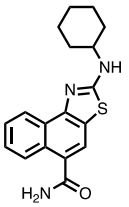
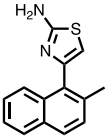
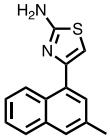
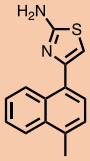
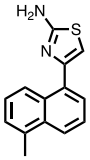
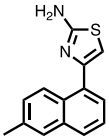
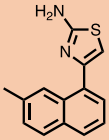
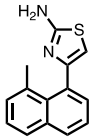
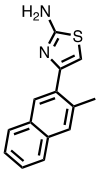
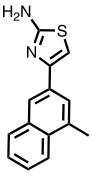
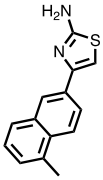
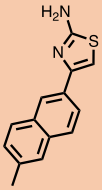
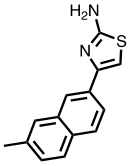
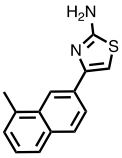
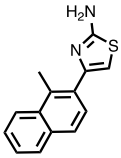
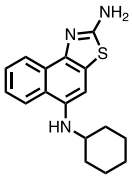
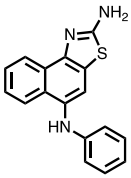
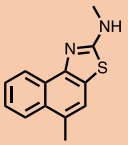
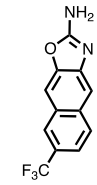
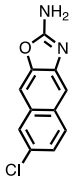
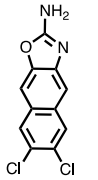
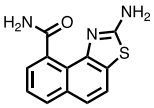
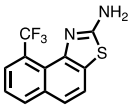
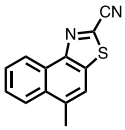
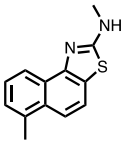
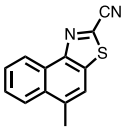
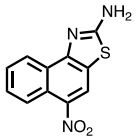
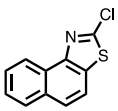
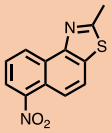
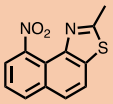
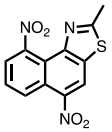
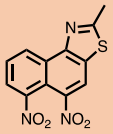
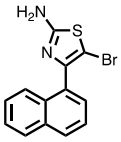
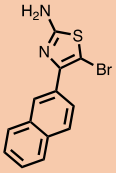
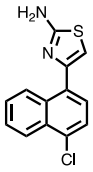
SKA-182 	SKA-183 	SKA-184 	SKA-185 	SKA-186 	SKA-187 	SKA-188 	SKA-189 
SKA-190 	SKA-191 	SKA-192 	SKA-193 	SKA-194 	SKA-195 	SKA-196 	SKA-197 
SKA-198 	SKA-199 	SKA-200 	SKA-201 	SKA-202 	SKA-203 	SKA-204 	SKA-205 
SKA-206 	SKA-207 	SKA-208 	SKA-209 	SKA-210 	SKA-211 	SKA-212 	SKA-213 
SKA-214 	SKA-215 	SKA-216 	SKA-217 	SKA-218 	SKA-219 	SKA-220 	SKA- 221 
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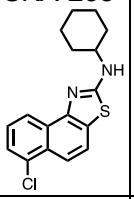
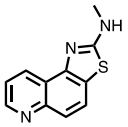
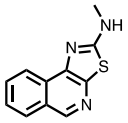
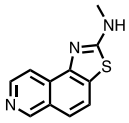
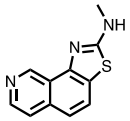
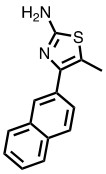
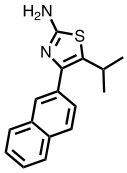
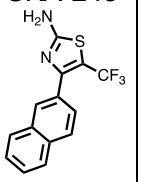
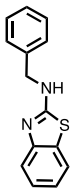
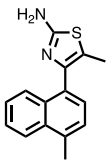
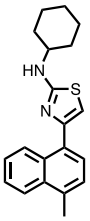
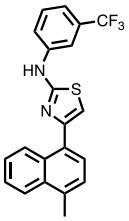
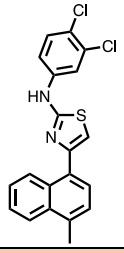
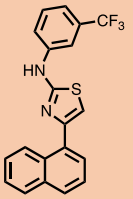
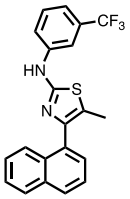
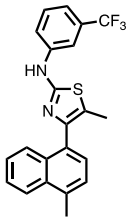
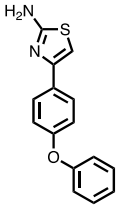
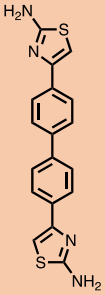
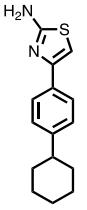
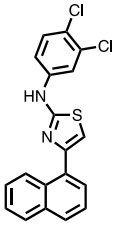
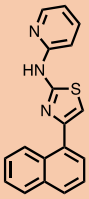
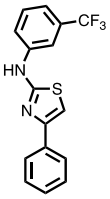
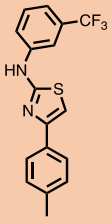
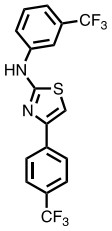
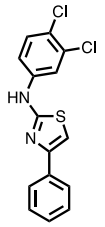
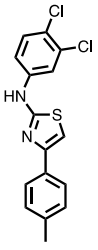
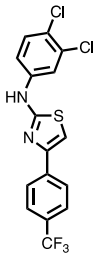
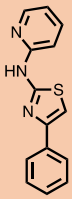
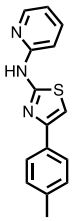
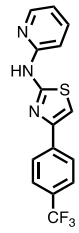
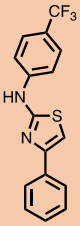
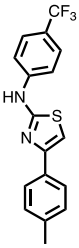
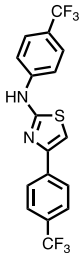
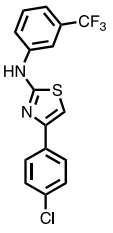
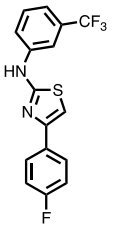
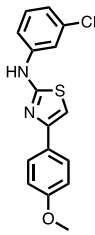
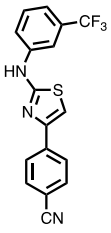
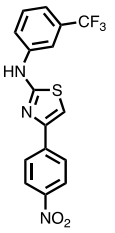
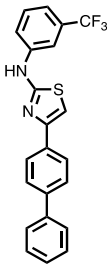
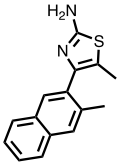
SKA-238 	SKA-239 	SKA-240 	SKA-241 	SKA-242 	SKA-243 	SKA-244 	SKA-245 
SKA-246 	SKA-247 	SKA-248 	SKA-249 	SKA-250 	SKA-251 	SKA-252 	SKA-253 
SKA-254 	SKA-255 	SKA-256 	SKA-257 	SKA-258 	SKA-259 	SKA-260 	SKA-261 
SKA-262 	SKA-263 	SKA-264 	SKA-265 	SKA-266 	SKA-267 	SKA-268 	SKA-269 
SKA-270 	SKA-271 	SKA-272 	SKA-273 	SKA-274 	SKA-275 	SKA-276 	SKA-277 
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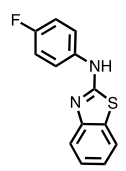
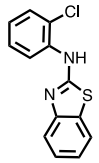
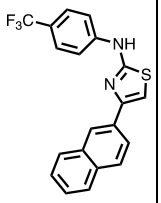
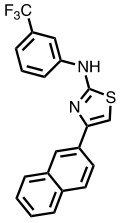
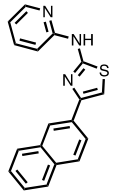
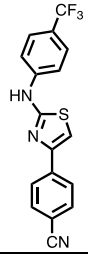
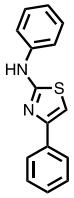
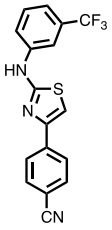
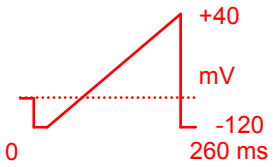
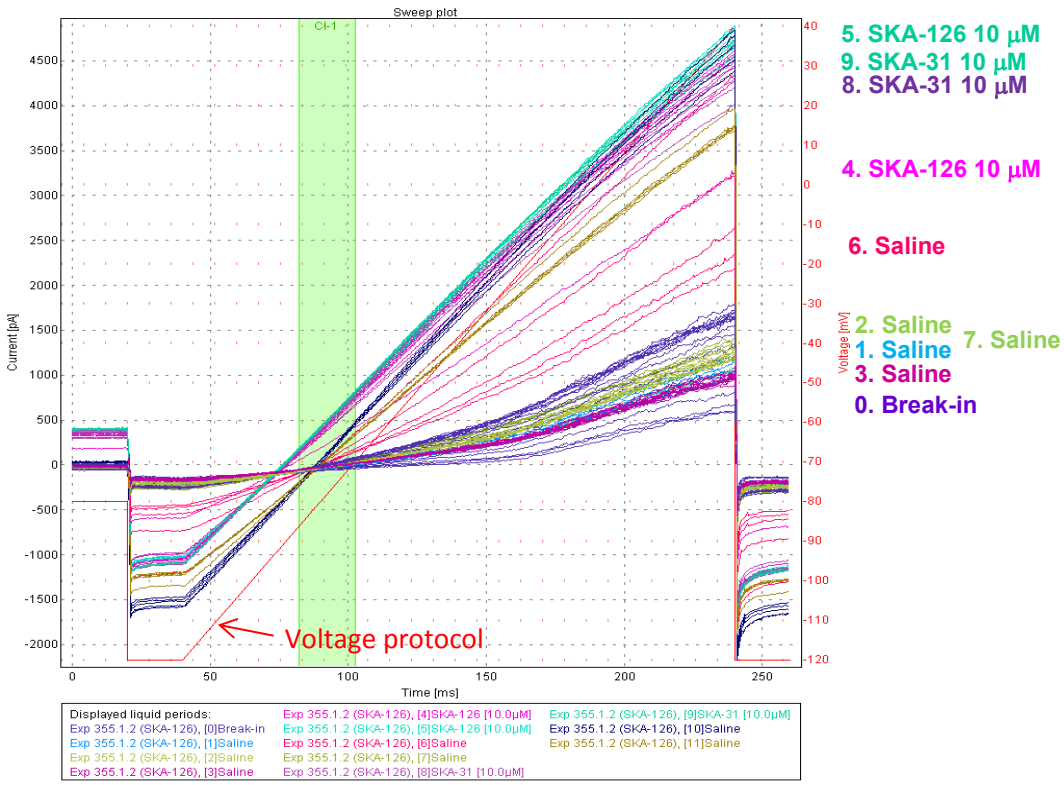
							

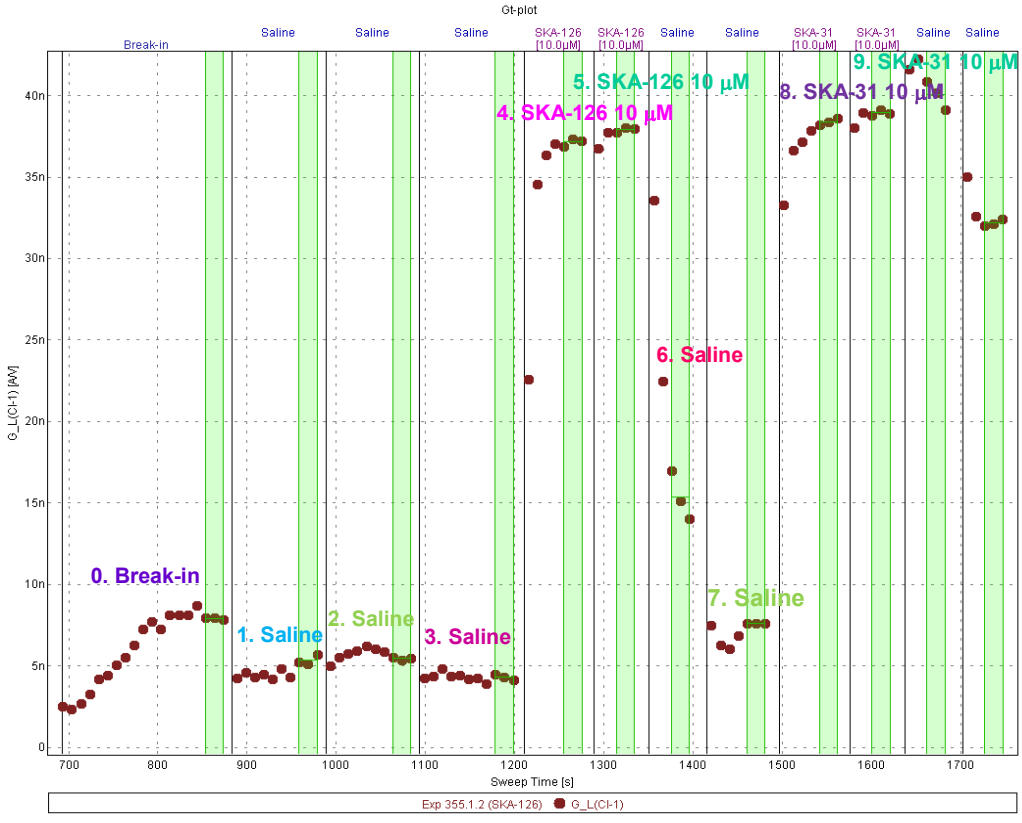
Supplementary Fig.3

QPatch screening of activator compounds on $K_{Ca}3.1$ and $K_{Ca}2.2$ channels with 250 nM of free Ca^{2+} in the internal solution.

Screening of SKA-126 on $K_{Ca}3.1$

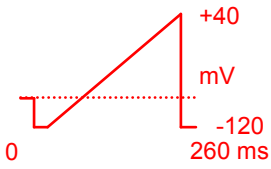


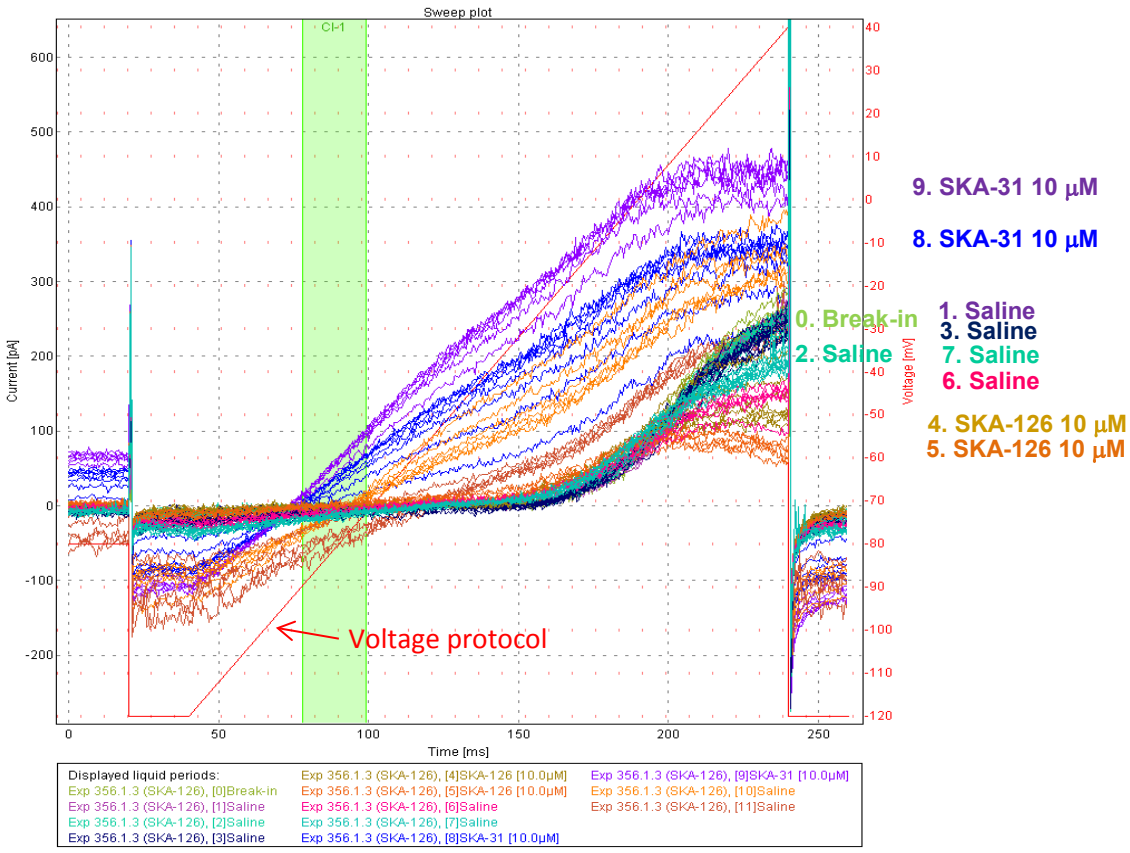


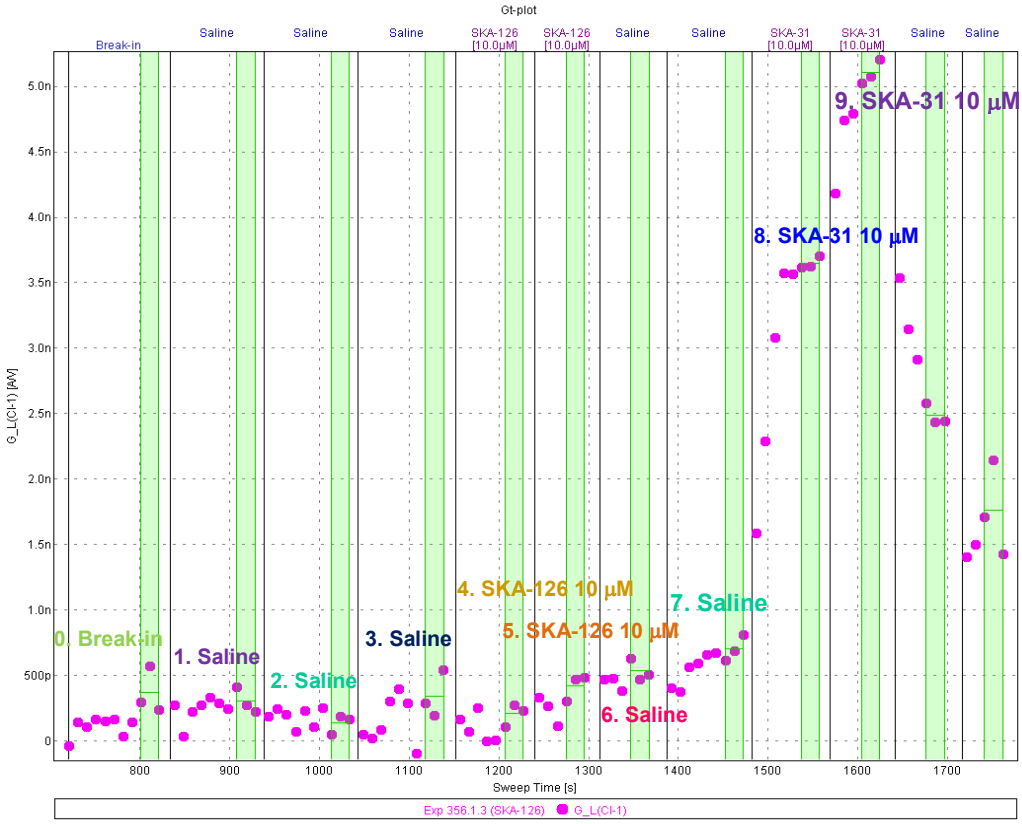


Raw current traces (top) and plot of current slope (measured between - 85 and - 65 mV) versus time for K_{Ca} activation (bottom). SKA-31 was used as the positive control. Saline or drugs were perfused during the first 15 sec of each so-called liquid period.

Screening of SKA-126 on $K_{Ca}2.2$

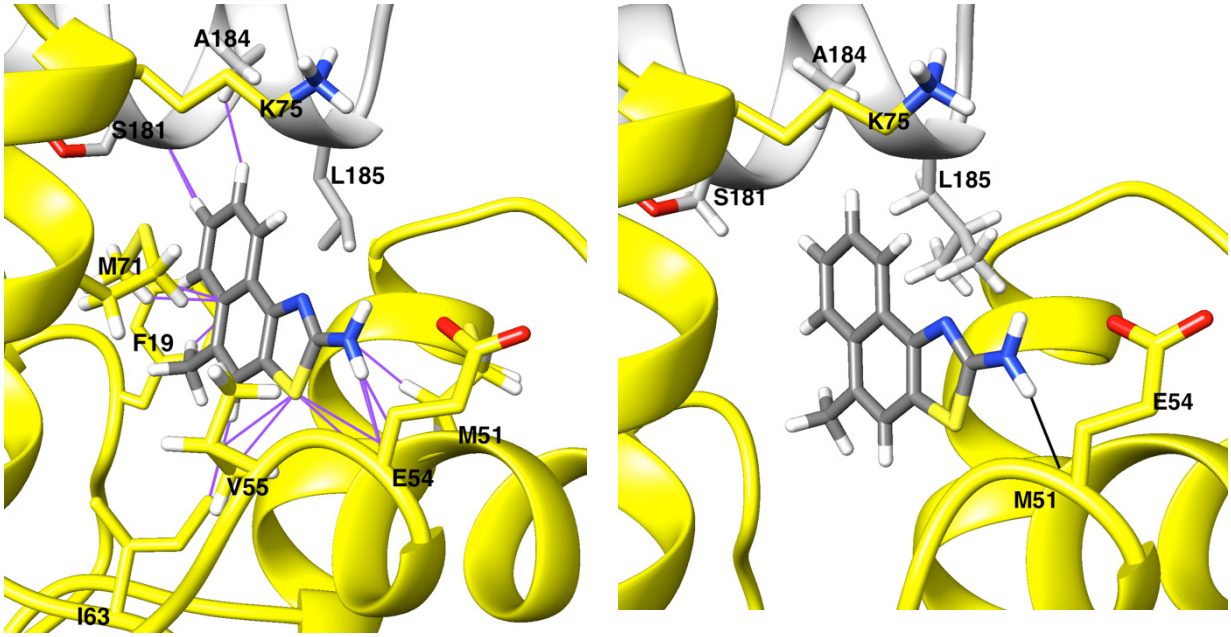






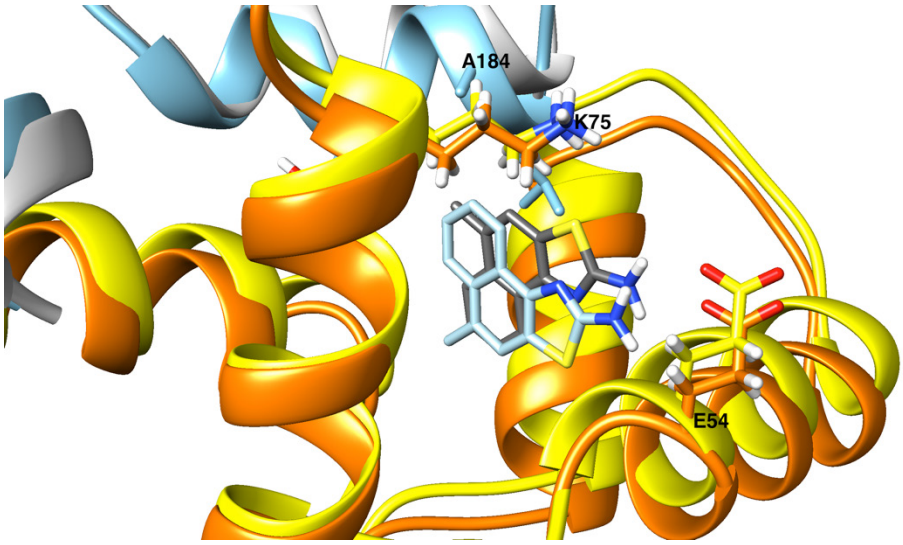
Raw current traces (top) and plot of current slope (measured between - 85 and - 65 mV) versus time for K_{Ca} activation (bottom). SKA-31 was used as the positive control.
Note: $K_{Ca}2.2$ currents are smaller than $K_{Ca}3.1$ currents and show some rectification at positive potentials.

Supplementary Fig.4



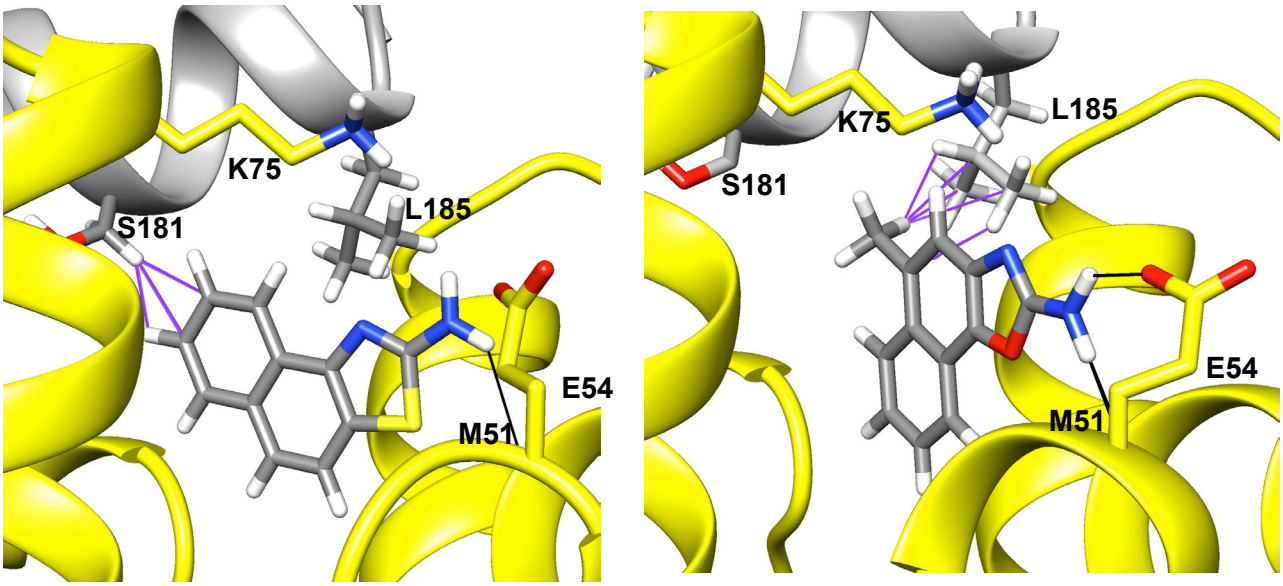
VDW interactions Hydrogen bonds

SKA-111 in K_{Ca}3.1 open state 2 (pdb id: 6cno)
The channel is shown in gray and the CaM N-lobe in yellow. Hydrogen bonds are shown in black, van der Waals interactions are visualized in purple. SKA-111 forms one hydrogen bond with M51 (right) and makes 12 van der Waals contacts with residues in the S_{45}A helix and the CaM N-lobe (left).



Overlay of the Rosetta docking models of SKA111 in open state 1 (yellow and light grey) and open state 2 (orange and sky blue). SKA-111 is shown in dark grey in open state 1 and in light blue in open state 2.

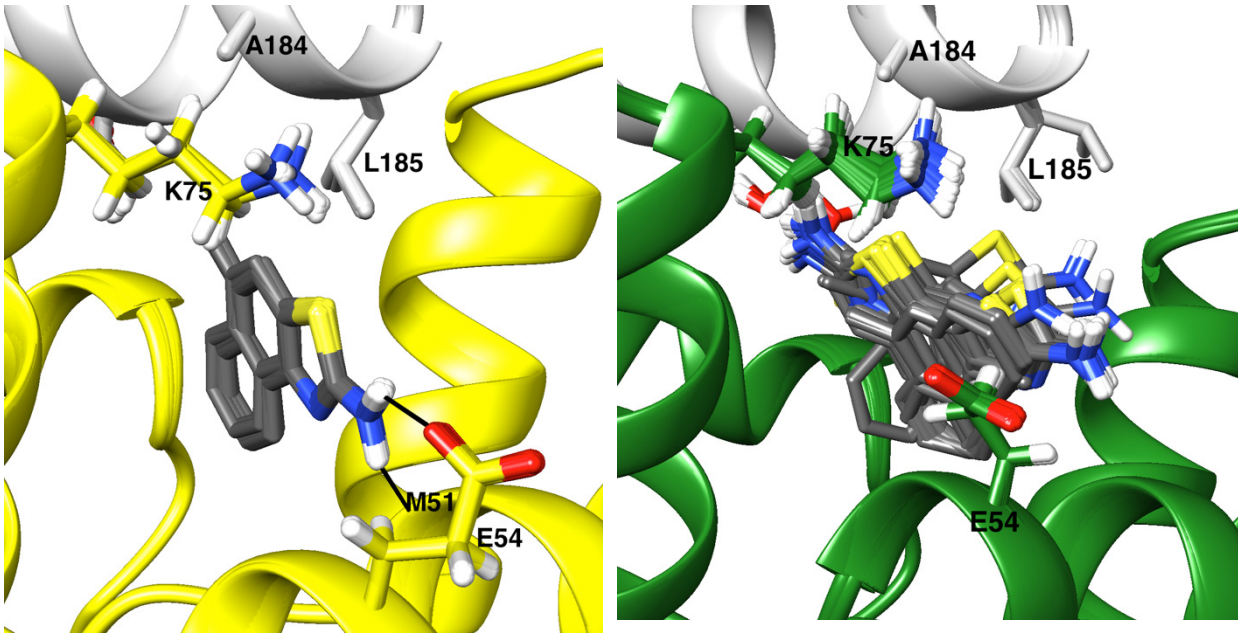
Supplementary Fig.5



SKA-31 (left) and SKA-121 (right) in K_{Ca}3.1 open state 1 (pdb id: 6cnn)

The channel is shown in gray and the CaM N-lobe in yellow. Hydrogen bonds are shown in black, van der Waals interactions are visualized in purple. For clarity, not all side chains of CaM residues within contact range of ligand are explicitly shown.

Supplementary Fig.6



SKA-111 in WT **S372R**

Rosetta ligand docking models of the top 50 lowest energy-binding poses of SKA-111 in the interface between the CaM N-lobe (yellow: WT, green: S372R) and the S_{45}A helix (light gray) of wild type KCa3.1 and the S372R K_{Ca}3.1 mutant. Molecular docking shows that SKA-111 converged well in wild type K_{Ca}3.1 but not in the S372R KCa3.1 mutant channel.