Supplemental material

```
Cav1.1 I      EWPWIYFVTLILLGSFFILNLVLGVLSGEFT
Cav1.2 I      ELPWVYFVSLVIFGSFFVLNLVLGVISGEFS
Cav1.3 I      ELPWVYFVSLVIFGSFFVLNLVLGVLSGEFS
Cav1.4 I      ELPWVYFVSLVIFGSFFVLNLVLGVLSGEFS
Cav2.1 I      TWNWLYFIPLIIIGSFFMLNLVLGVLSGEFA
Cav2.2 I      TWNWLYFIPLIIIGSFFMLNLVLGVLSGEFA
Cav2.3 I      TWNWLYFIPLIIIGSFFVLNLVLGVLSGEFA
Cav3.1 I      FYNFIYFILLIIVGSFFMINLCLVVIATQFS
Cav3.2 I      FYNFIYFILLIIVGSFFMINLCLVVIATQFS
Cav3.3 I      FYNFIYFILLIIVGSFFMINLCLVVIATQFS
Cav1.1 II     MLVCIYFIILFVCGNYILLNVFLAIAVDNLA
Cav1.2 II     MLVCIYFIILFICGNYILLNVFLAIAVDNLA
Cav1.3 II     MIVCIYFIILFICGNYILLNVFLAIAVDNLA
Cav1.4 II     MLVCIYFIILFICGNYILLNVFLAIAVDNLA
Cav2.1 II     MVFSIYFIVLTLFGNYTLLNVFLAIAVDNLA
Cav2.2 II     MFSSFYFIVLTLFGNYTLLNVFLAIAVDNLA
Cav2.3 II     MWSAIYFIVLTLFGNYTLLNVFLAIAVDNLA
Cav3.1 II     SWAALYFIALMTFGNYVLFNLLVAILVEGFQ
Cav3.2 II     SWAALYFIALMTFGNYVLFNLLVAILVEGFQ
Cav3.3 II     PWASLYFVALMTFGNYVLFNLLVAILVEGFQ
Cav1.1 III    VEMAIFFIIYIILIAFFMMNIFVGFVIVTFQ
Cav1.2 III    VEISIFFIIYIIIIAFFMMNIFVGFVIVTFQ
Cav1.3 III    VEISIFFIIYIIIVAFFMMNIFVGFVIVTFQ
Cav1.4 III    VEISVFFIVYIIIIAFFMMNIFVGFVIITFR
Cav2.1 III    MEMSIFYVVYFVVFPFFFVNIFVALIIITFQ
Cav2.2 III    MELSIFYVVYFVVFPFFFVNIFVALIIITFQ
Cav2.3 III    MEMSIFYVVYFVVFPFFFVNIFVALIIITFQ
Cav3.1 III    PWMLLYFISFLLIVAFFVLNMFVGVVVENFH
Cav3.2 III    PWMLLYFISFLLIVSFFVLNMFVGVVVENFH
Cav3.3 III    PWMLLYFISFLLIVSFFVLNMFVGVVVENFH
Cav1.1 IV     NFAYYYFISFYMLCAFLVINLFVAVIMDNFD
Cav1.2 IV     SFAVFYFISFYMLCAFLIINLFVAVIMDNFD
Cav1.3 IV     NFAIVYFISFYMLCAFLIINLFVAVIMDNFD
Cav1.4 IV     NFAIAYFISFFMLCAFLIINLFVAVIMDNFD
Cav2.1 IV     EFAYFYFVSFIFLCSFLMLNLFVAVIMDNFE
Cav2.2 IV     DFAYFYFVSFIFLCSFLMLNLFVAVIMDNFE
Cav2.3 IV     DLAYVYFVSFIFFCSFLMLNLFVAVIMDNFE
Cav3.1 IV     VISPIYFVSFVLTAQFVLVNVVIAVLMKHLE
Cav3.2 IV     ALSPVYFVTFVLVAQFVLVNVVVAVLMKHLE
Cav3.3 IV     FVSPLYFVSFVLTAQFVLINVVVAVLMKHLD
```

Fig. S1. Alignment of S6 helices from all ten human Ca_V channels

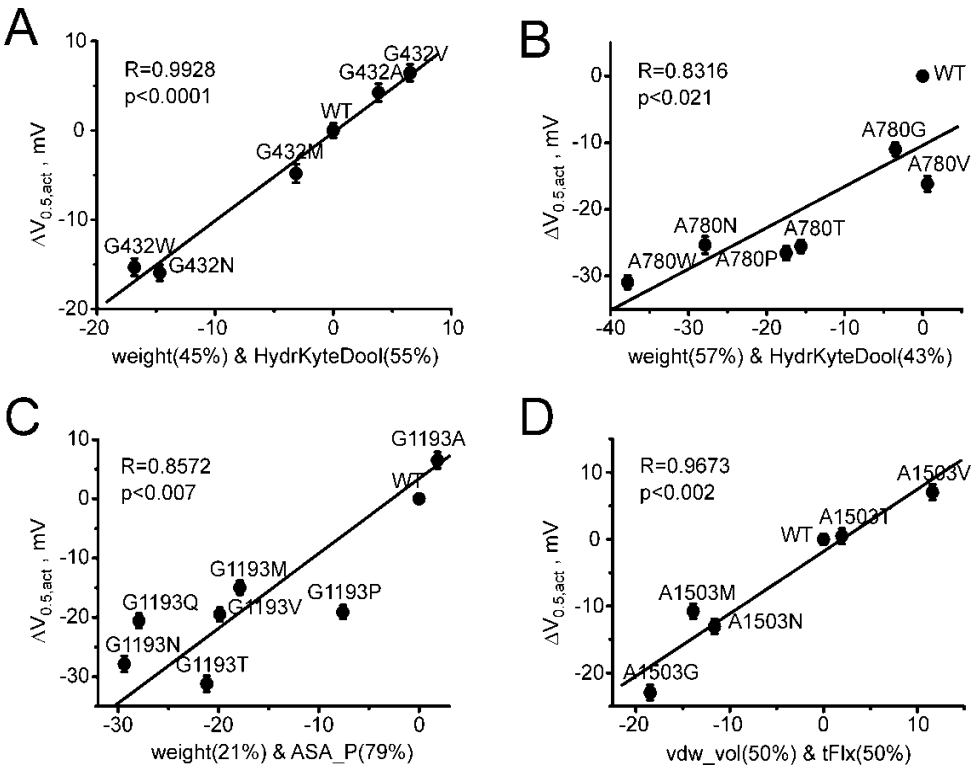

Fig. S2. Role of residue size in channel activation in S6 segments of $Ca_V1.2$.
Correlation between the shifts of the activation curves (ΔV act) and a linear combination of descriptors characterizing the size of amino acids (either molecular weight (A, B, C) or Van der Waals volume[1] (D)) in combination with hydrophobicity indices such as HydrKyteDool[2] or ASA_P[3] (A, B, C) or a flexibility index[4] (D) with the shifts of the activation curve.

[1] vdw_vol – Van der Waals volume
[2] HydrKyteDool – hydrophobicity (1)
[3] ASA_P – Water accessible surface area of all polar atoms. Accessible surface area refers to the water accessible surface area using a probe radius of 1.4 Angstroms.
[4] tFlx - Amino acid side chain flexibility (2)

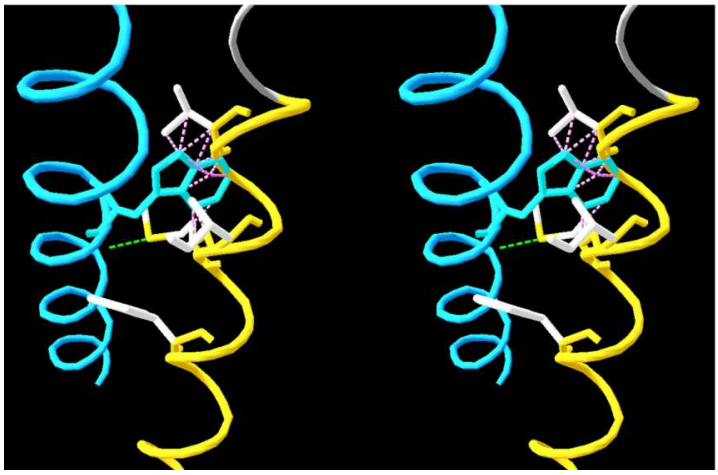

Fig. S3. Schematic illustration of packing distortions due to insertion of bulky hydrophobic residues in position G432. Stereo view of helices IS6 (cyan) neighboring IVS6 (yellow). G432 was mutated to W432 using Deep View software (3). Unfavorable steric interactions are shown as pink dots.

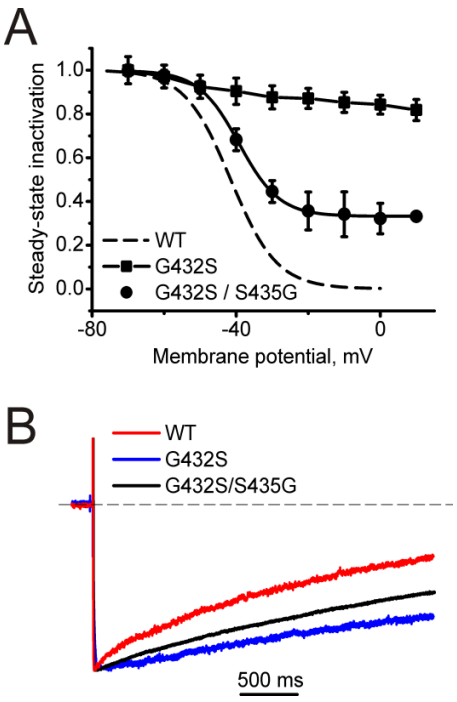

Fig. S4. Mutation S435G partially rescue channel inactivation.
A, Averaged inactivation curves of double mutation G432S/S435G in comparison with wild-type.
B, Representative I_{Ba} through wild-type and indicated mutant channels during depolarizing test pulses from −100 mV to the peak potentials of the current-voltage relationships (see Table 1 for mean r_{3000} and $V_{0.5,inact}$).

References

1. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol* **157**, 105-132
2. Gottfries, J., and Eriksson, L. (2010) *Mol. Divers* **14**, 709-718
3. Guex, N. and Peitsch, M.C. (1997) *Electrophoresis* **18**, 2714-2723