

Supplemental Data

Nitric Oxide Supplementation for Treatment of

Long-Term Complications in Argininosuccinic Aciduria

Sandesh C.S. Nagamani, Philippe M. Campeau, Oleg Shchelochkov, Muralidhar H. Premkumar, Kilian Guse, Nicola Brunetti-Pierri, Yuqing Chen, Qin Sun, Yaoping Tang, Donna Palmer, Anilkumar K Reddy, Li Li, Timothy C. Slesnick, Daniel I. Feig, Susan Caudle, David Harrison, Leonardo Salviati, Juan C. Marini, Nathan S. Bryan, Ayelet Erez, and Brendan Lee

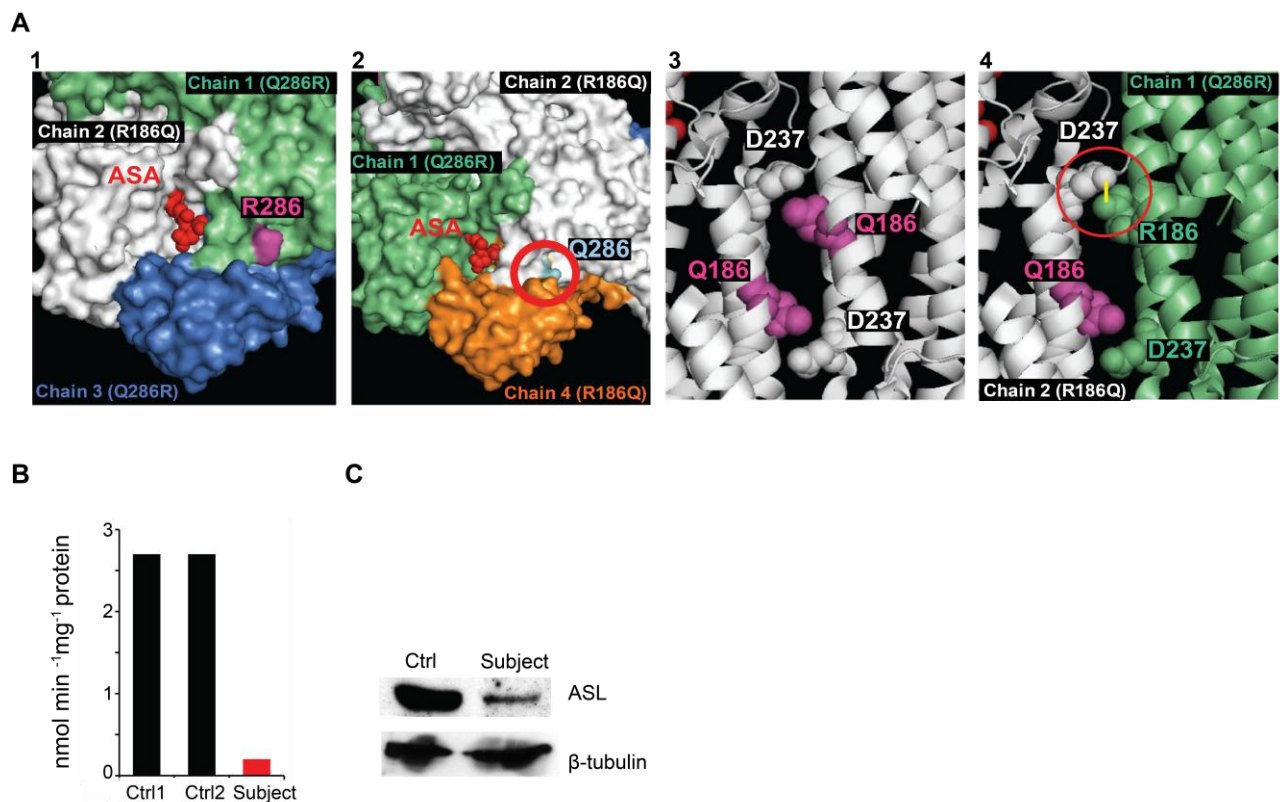


Figure S1. Mutation Analysis and Enzyme Activity Show Residual ASL Activity in the ASA Subject with Hypertension

(A) 3D model of the ASL heterotetramer composed of two chains (1 and 3) with the Q286R mutation and two chains (2 and 4) with the R186Q mutation. A1. Interaction

between chains (1 and 3) carrying the Q286R mutation (magenta residue), interferes with ASL catalytic site thus predicted to reduce enzymatic activity. A2. The conformation of the heterotetramer (2- Q286R, 2- R186Q), allows at least one normal active site (red circle, with no magenta residue) to be formed thus providing allelic complementation. A3. In the wild type context, R186 physically interacts with D237 on the opposite monomer to stabilize the protein; the homozygous substitution R186Q prevents this interaction. A4. Presence of Q286R allele and the R186Q allele in the ASL heterotetramer, allows for at least one functional D237-R186 interaction to be formed (red circle), which results in residual enzyme activity.

(B) Enzyme activity performed on the skin fibroblasts shows that the subject has 10% activity of controls.

(C) Western blot on the fibroblasts showing decreased ASL protein expression that correlates with the decreased enzyme activity.

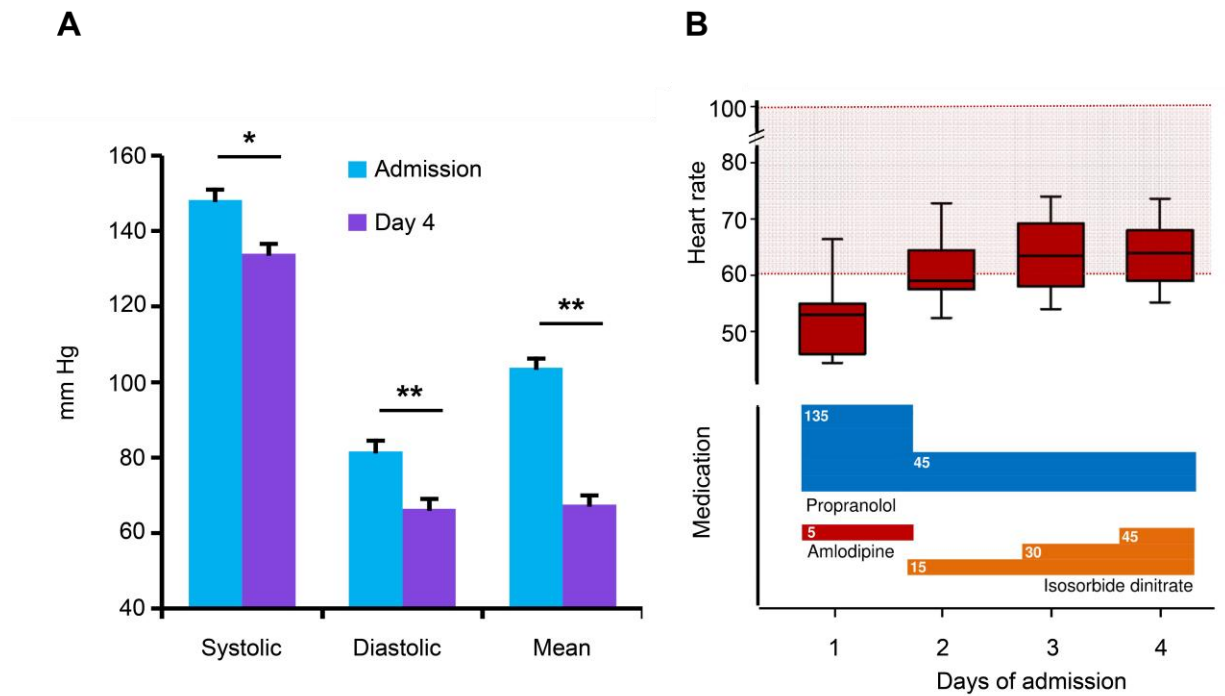


Figure S2. Hospital Course of the ASA Subject with Hypertension

(A) Blood pressure measurements during hospitalization show significant decreases in the systolic, diastolic and mean arterial blood pressures on day 4 after initiation of nitrate therapy (* $p < 0.05$, ** $p < 0.005$).

(B) The median values of the heart rate during hospitalization show normalization of the heart rate with the weaning of propranolol. The bradycardia noted prior to decrease in beta blockade implies compliance with the medication. (Box plots depict the 10th, 25th, 75th and the 90th centiles).