SUPPLEMENTAL INFORMATION

Table S1. **Bodyweight for aged rats.** The bodyweights of cage control rats, or rats that underwent 14 days of HS or 14 days of recovery following HS are presented as mean ± SD. Cage control-vehicle treated (CC-Veh) and cage control-HMB (CC-HMB) treated animals did not receive hindlimb suspension but were examined at the same time points as the other animals.

Table S2. **Isometric force/bodyweight for aged rats.** The plantarflexor isometric force/bodyweight ratio of rats is reported in each group as Newtons of force/gram bodyweight (mean ± SD). The conditions include: after cage control conditions, after 14 days of HS or 14 days of recovery following HS. Cage control-vehicle treated (CC-Veh) and cage control-HMB (CC-HMB) treated animals did not receive hindlimb suspension but were examined at the same time points as the other animals.

Table S3. **Gastrocnemius muscle wet weight for aged animals.** Gastrocnemius muscle wet weight data (mean ± SD) are summarized from aged animals that were used for the current study under control conditions, or after 14 days of HS or 14 days of recovery following HS. Cage control-vehicle treated (CC-Veh) and cage control-HMB (CC-HMB) treated animals did not receive hindlimb suspension but were examined at the same time points as the other animals. Gastrocnemius wet weight was not significantly reduced by HS or HS+ recovery.

Table S4. **Bodyweight for young adult rats.** The bodyweight of young adult rats (12 months of age) were obtained before HS, after 14 days of HS or after 14 days of recovery following HS. The dietary and control conditions were the same as used for the aged animals. The conditions are after cage control conditions, after 14 days of HS or 14 days of recovery following HS. Cage control-vehicle treated (CC-Veh) and cage control-HMB (CC-HMB) treated animals did not receive hindlimb suspension but were examined at the same time points as the other animals. Values are means ± SD. Time 0, before hindlimb suspension; 14d HS, 14 days after hindlimb suspension; Recover, after 14 days of HS followed by 14 days of reloading. $^{\S}p<0.05$, compared to Pre-HS.* $p<0.05$, compared to Post-HS.

Table S5. **Isometric force/bodyweight for young adult rats.** The plantarflexor isometric force/bodyweight ratio for young adult rats (12 months) in each group is reported as Newtons of force/gram bodyweight. The conditions are: after cage control conditions, after 14 days of HS or 14 days of recovery following HS. Cage control-vehicle treated (CC-Veh) and cage control-HMB (CC-HMB) treated animals did not receive hindlimb suspension but were examined at the same time points as the other animals. Values are means ± SD. Time 0, before hindlimb suspension; 14d HS, 14 days after hindlimb suspension; Recover, after 14 days of HS followed by 14 days of reloading.

Figure S1. **Muscle wet weight data for young animals subjected to hindlimb suspension or after recovery following unloading.** Muscle wet weight was obtained in plantaris (**A**) and soleus (**C**) muscles of young adult rats (12 months of age) control animals for the hindlimb suspension group (HS Con), the recovery

group (R Con), and in experimental animals after 14 days of hindlimb suspension (HS) or after 14 days of hindlimb suspension followed by 14 days of reloading (R). The ratio of muscle wet weight to bodyweight is presented for the plantaris (**B**) and the soleus **(D)** muscles of the aged animals after each condition. The animals received HMB or the vehicle (water) daily by gavage, for a total of 21 days (HS Con and HS) or for 32 days (R Con and R). An ANOVA followed by Bonferroni post hoc analyses was used to evaluate the differences between the group mean. †, $p<0.05$, HS vs. R animals or control animals for that experimental condition. Six animals were examined in each group. There was no effect of HMB on muscle wet weight responses to loading or recovery.

Figure S2. **The apoptotic index.** The apoptotic index was calculated from tissue cross sections of the plantaris muscle (**A**) and the soleus muscle (**B**) of young adult FBN rats (12 months of age) by determining the number ratio of TUNEL positive nuclei to total nuclei in tissue cross sections. The muscle groups were: the cage control animals for the hindlimb suspension group (HS Con), the cage control animals for the recovery group (R Con), and in experimental animals after 14 days of hindlimb suspension (HS) or after 14 days of hindlimb suspension followed by 14 days of reloading (R). Only nuclei that were directly below or touching the basal lamina were counted. The animals received either HMB or the vehicle (water) daily by gavage, for a total of 21 days (HS Con and HS) or for 32 days (R Con and R). An ANOVA followed by Bonferroni post hoc analyses was used to evaluate the differences between the group means. †, $p<0.05$, HS vs. R animals or control animals for that experimental condition. Six animals were examined in each group. The data are presented as mean ± SD.

Figure S3. **Apoptotic signaling proteins in the plantaris muscle of young adult rats.** The protein abundance was determined by western blots in the plantaris muscle of young adult (12 months of age) FBN rats for Bax (**A**), Bcl-2 (**B**), cleaved caspase-9 (**C**), and cleaved caspase-3 (**D**). The groups include: controls for the hindlimb suspension group (HS Con), controls for the recovery group (R Con), and in experimental animals after 14 days of hindlimb suspension (HS) or after 14 days of hindlimb suspension followed by 14 days of reloading (R). The animals received HMB or the vehicle (water) daily by gavage, for a total of 21 days (HS Con and HS) or for 32 days (R Con and R). Six animals were examined in each group. α-tubulin was used as a loading control. The data were normalized to α-tubulin and were expressed as mean ± SD. An ANOVA followed by Bonferroni post hoc analyses was used to evaluate the differences between the group means. †, $p<0.05$, HS vs. R animals or control animals for that experimental condition. All apoptotic markers had returned to control levels in the R group. HMB had no effect on apoptotic signaling in the plantaris muscles of young adult rats.

Figure S4. **Apoptotic signaling proteins in the soleus muscle of young adult rats.** The protein abundance was determined by western blots in the soleus muscle of young adult (12 months of age) FBN rats for Bax (**A**), Bcl-2 (**B**), cleaved caspase-9 (**C**), and cleaved caspase-3 (**D**). The groups include: controls for the hindlimb suspension group (HS Con), controls for the recovery group (R Con), and in experimental animals after 14 days of hindlimb suspension (HS) or after 14 days of hindlimb suspension followed by 14 days of reloading (R). The animals received HMB or the vehicle (water) daily by gavage, for a total of 21 days (HS Con and HS) or for 32 days (R Con and R). Six animals were in each

diet and experimental group. α-tubulin was used as a loading control. The data were normalized to α-tubulin and were expressed as mean ± SD. An ANOVA followed by Bonferroni post hoc analyses was used to evaluate the differences between the group means. †, $p<0.05$, HS vs. R animals or control animals for that experimental condition. All apoptotic markers had returned to control levels in the R group. HMB had no effect on apoptotic signaling in the plantaris muscles of young adult rats.