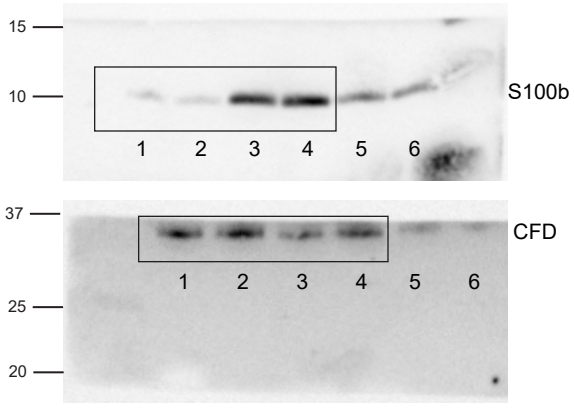
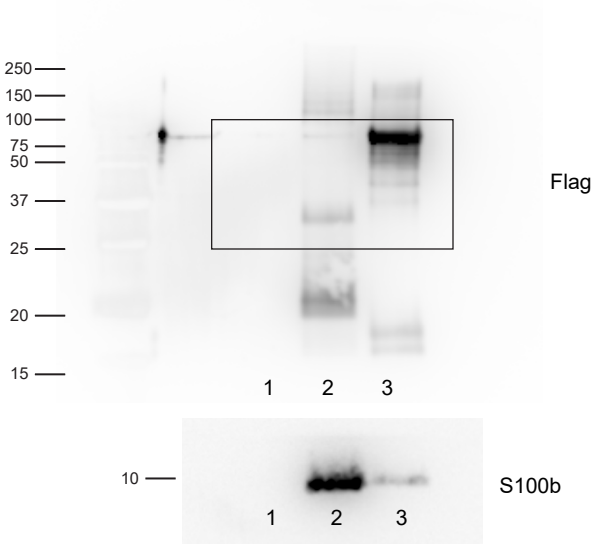
Figure 5i



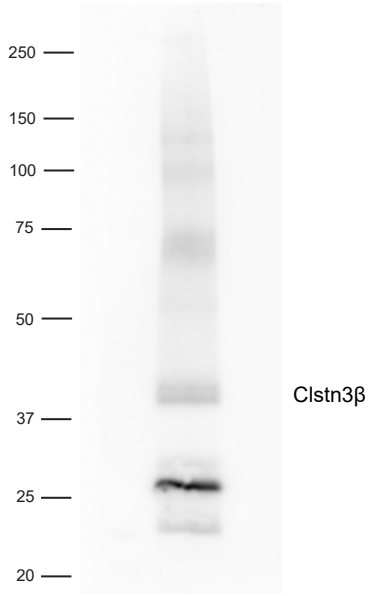
From left to right:
Lane 1: AdS100b alone rep1
Lane 2: AdS100b alone rep2
Lane 3: AdS100b+AdClstn3β rep1
Lane 4: AdS100b+AdClstn3β rep 2
Lane 5: 1/2 loading of Lane 3 to check dynamic range
Lane 6: 1/2 loading of lane 4 to check dynamic range

Figure 5j

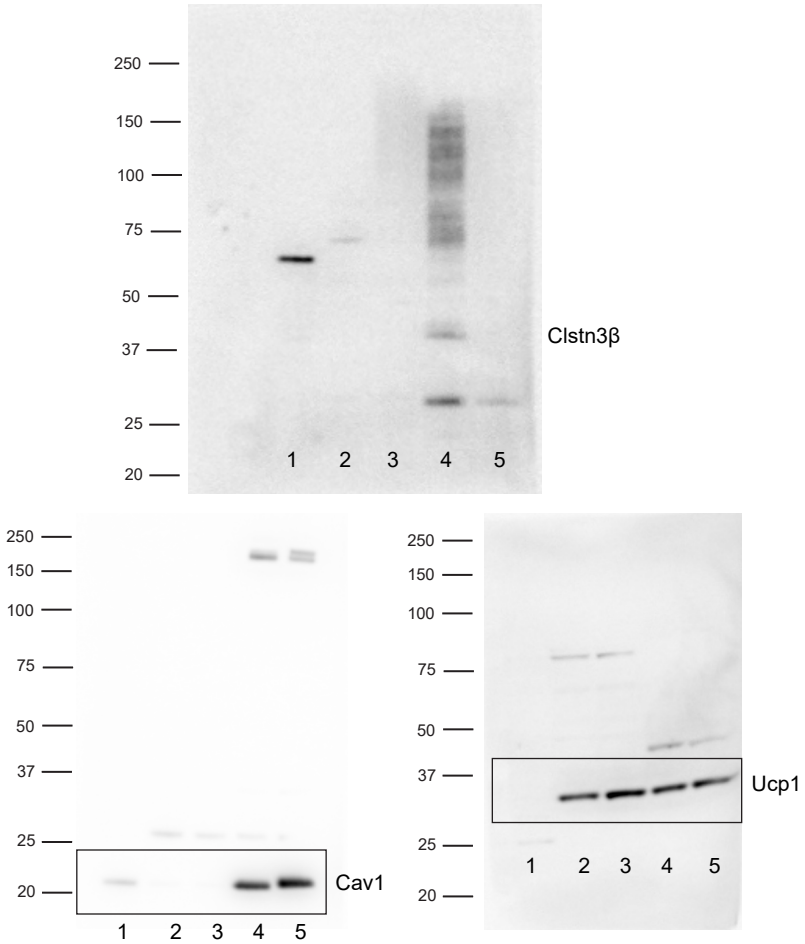


From left to right:
Lane 1: FLAG pull down from cells transfected with pcDNA
Lane 2: FLAG pull down from cells transfected with Clstn3β-Flag
Lane 3: FLAG pull down from cells transfected with Clstn3-Flag
Note in lane 2 the FLAG antibody detected various forms of Clstn3β that are also observed with the endogenous protein.

Extended Data Figure 1d



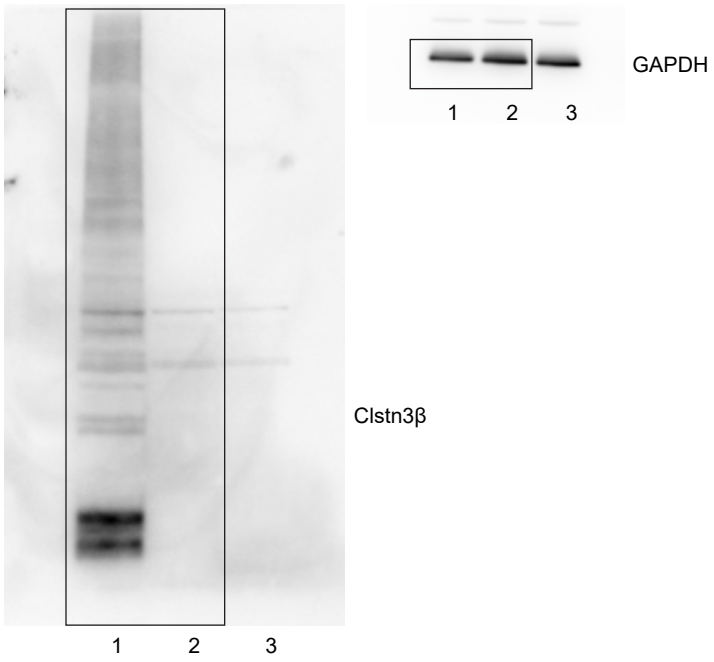
Extended Data Figure 2c



From left to right:
Lane 1: Cytoplasm
Lane 2: Nuclei
Lane 3: Mitochondria
Lane 4: Microsome
Lane 5: Plasma membrane

Note that Ucp1 appears in every fraction, although it is slightly more abundant in the mitochondrial fraction than other fractions. This is because mitochondria are extremely abundant in the brown adipose tissue from cold acclimated mice and it is technically impossible to separate mitochondria from other fractions cleanly. Nevertheless, the separation of other fractions is reasonably well, as indicated by Cav1 blot, which is expected to be present in the plasma membrane and microsome fractions.

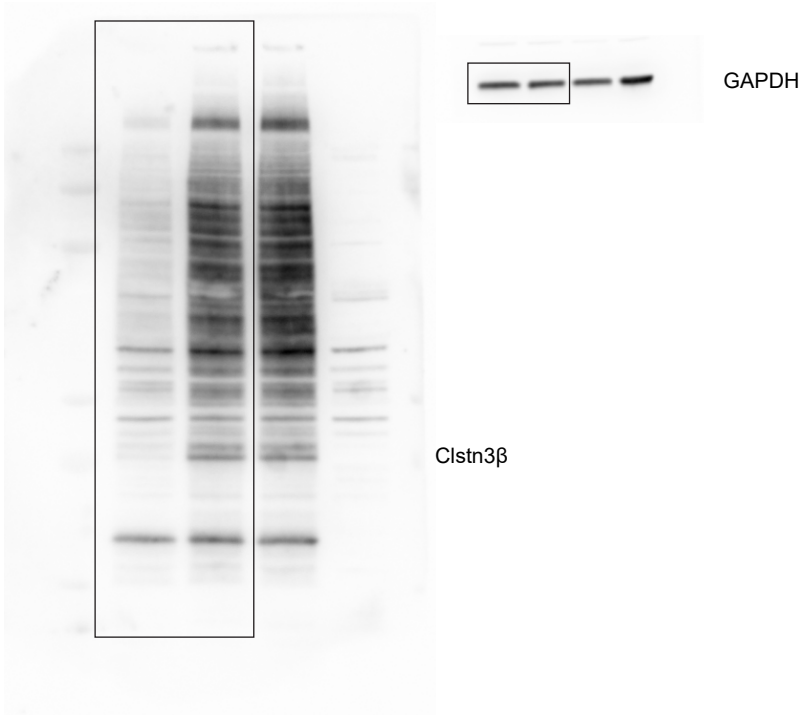
Extended Data Figure 3b



From left to right:
Lane 1: WT brown adipocytes
Lane 2: KO brown adipocytes 1
Lane 3: KO brown adipocytes 2

Lane 2 and 3 are preps of isolated brown adipocytes from different founders generated by CRISPR-Cas9 deletion of Clstn3β. They have different sequences at the deletion site but are all null alleles. The loading control was run on a separate gel because the whole gel was blotted for Clstn3β to visualize all forms of different molecular weights.

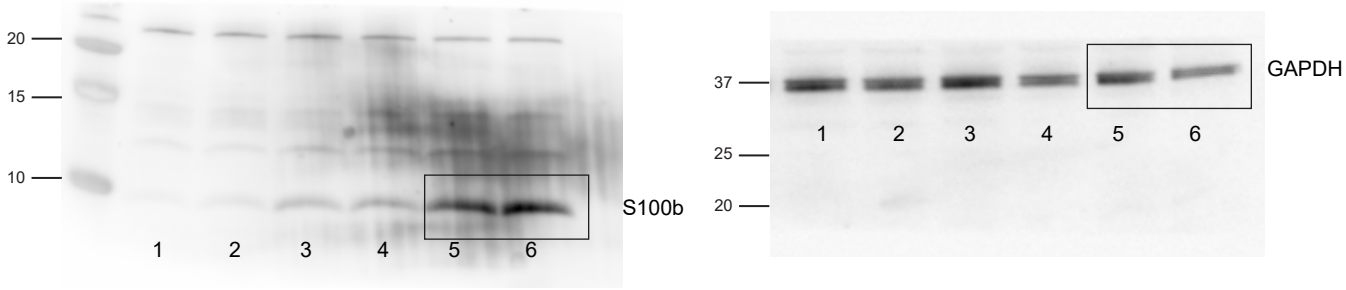
Extended Data Figure 4a



From left to right:
Lane 1: WT brown adipose tissue
Lane 2: TG brown adipose tissue 1
Lane 3: TG brown adipose tissue 2
Lane 4: KO brown adipose tissue

Lane 2 and 3 are preps of brown adipose tissue from different founders of the transgenic line. Note whole tissue rather than isolated adipocytes was used, compared to Extended Data Figure 3b. This is because transgenic brown adipocytes have too low lipid content to float upon centrifugation, which makes them difficult to isolate. Note that the use of whole tissue instead of isolated cells also yielded a different pattern of nonspecifc bands. The loading control was run on a separate gel because the whole gel was blotted for S100b.

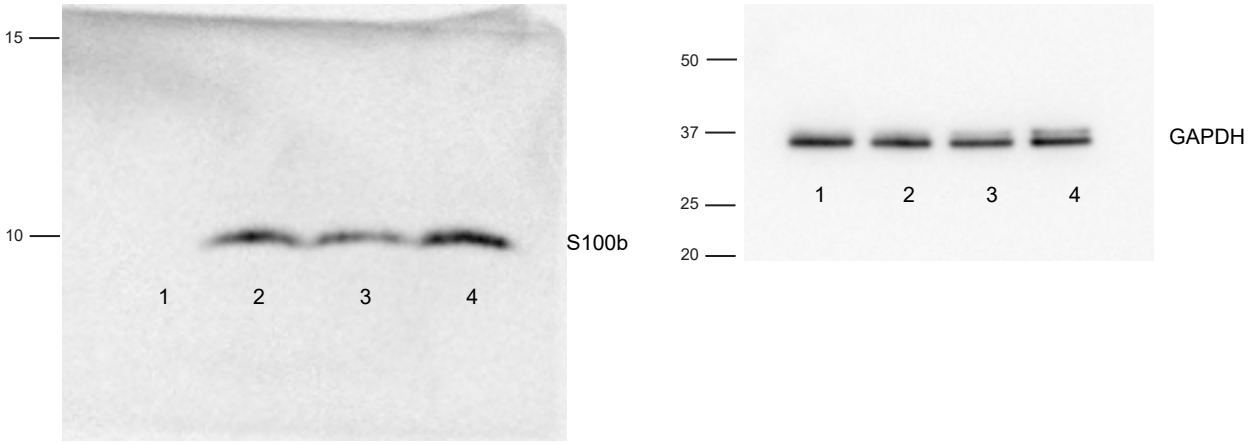
Extended Data Figure 5i



From left to right:
Lane 1: low titre AdS100b
Lane 2: low titre AdS100b+AdClstn3β
Lane 3: medium titre AdS100b
Lane 4: medium titre AdS100b+AdClstn3β
Lane 5: high titre AdS100b
Lane 6: high titre AdS100b +AdClstn3β

The loading control was run on a separate gel because the whole gel was blotted for S100b.

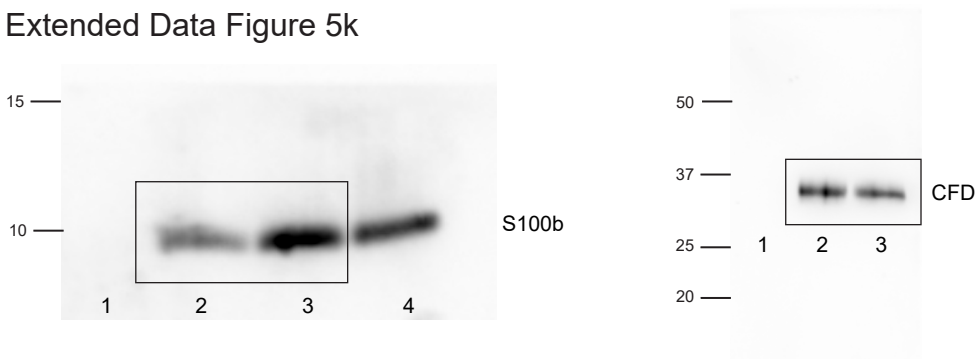
Extended Data Figure 5j



From left to right:
Lane 1: HEK293T cells transfected with pcDNA
Lane 2: HEK293T cells transfected with s100b + pcDNA
Lane 3: HEK293T cells transfected with s100b + clstn3
Lane 4: HEK293T cells transfected with s100b + clstn3β

Loading control was run on the same gel.

Extended Data Figure 5k



From left to right:
Lane 1: HEK293T cells transfected with pcDNA
Lane 2: HEK293T cells transfected with s100b + pcDNA
Lane 3: HEK293T cells transfected with s100b + clstn3
Lane 4: HEK293T cells transfected with s100b + clstn3β

From left to right:
Lane 1: HEK293T cells transfected with pcDNA
Lane 2: HEK293T cells transfected with cfd + pcDNA
Lane 3: HEK293T cells transfected with cfd + clstn3β

Supplementary Figure 1. Gel source data