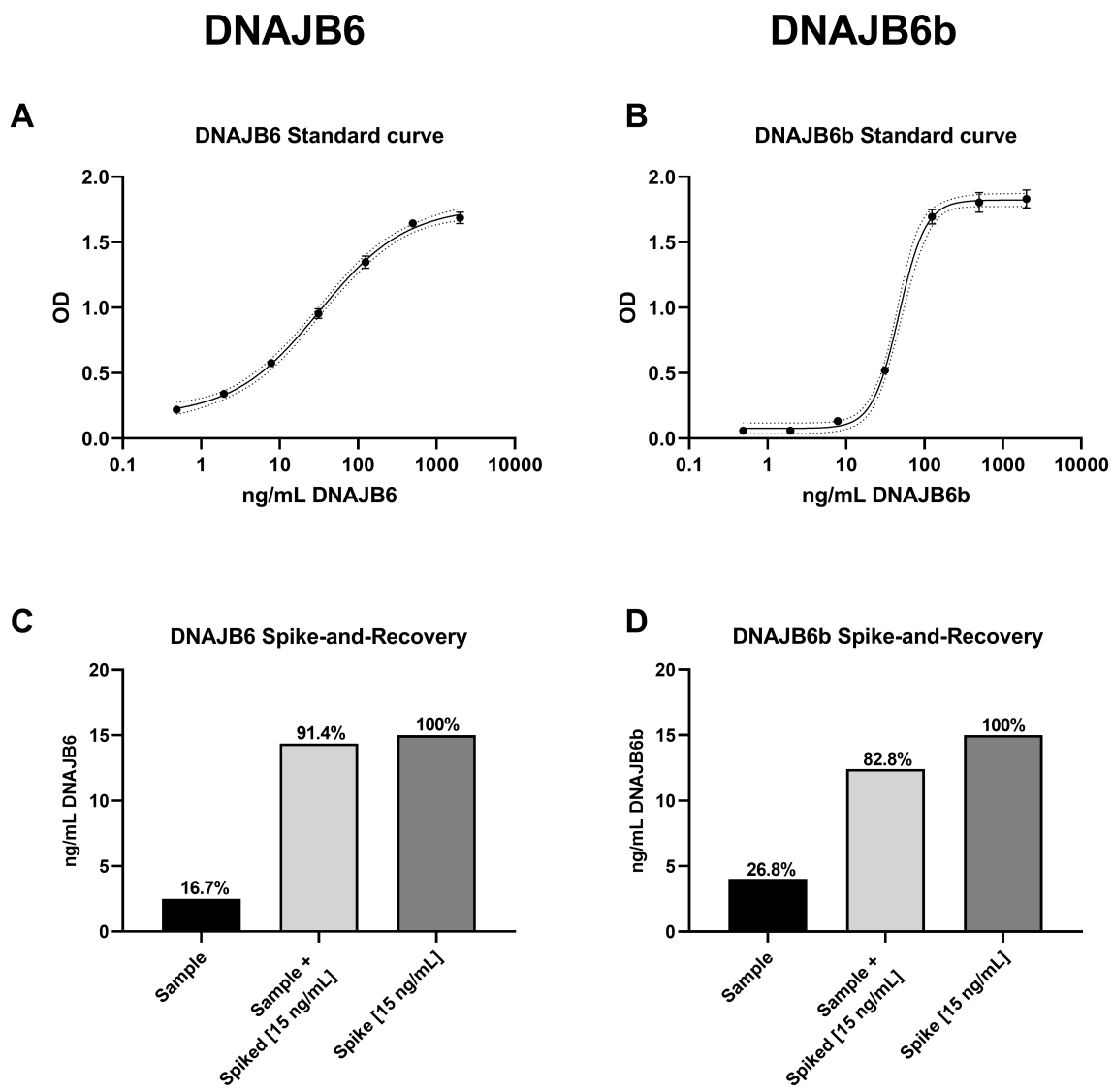
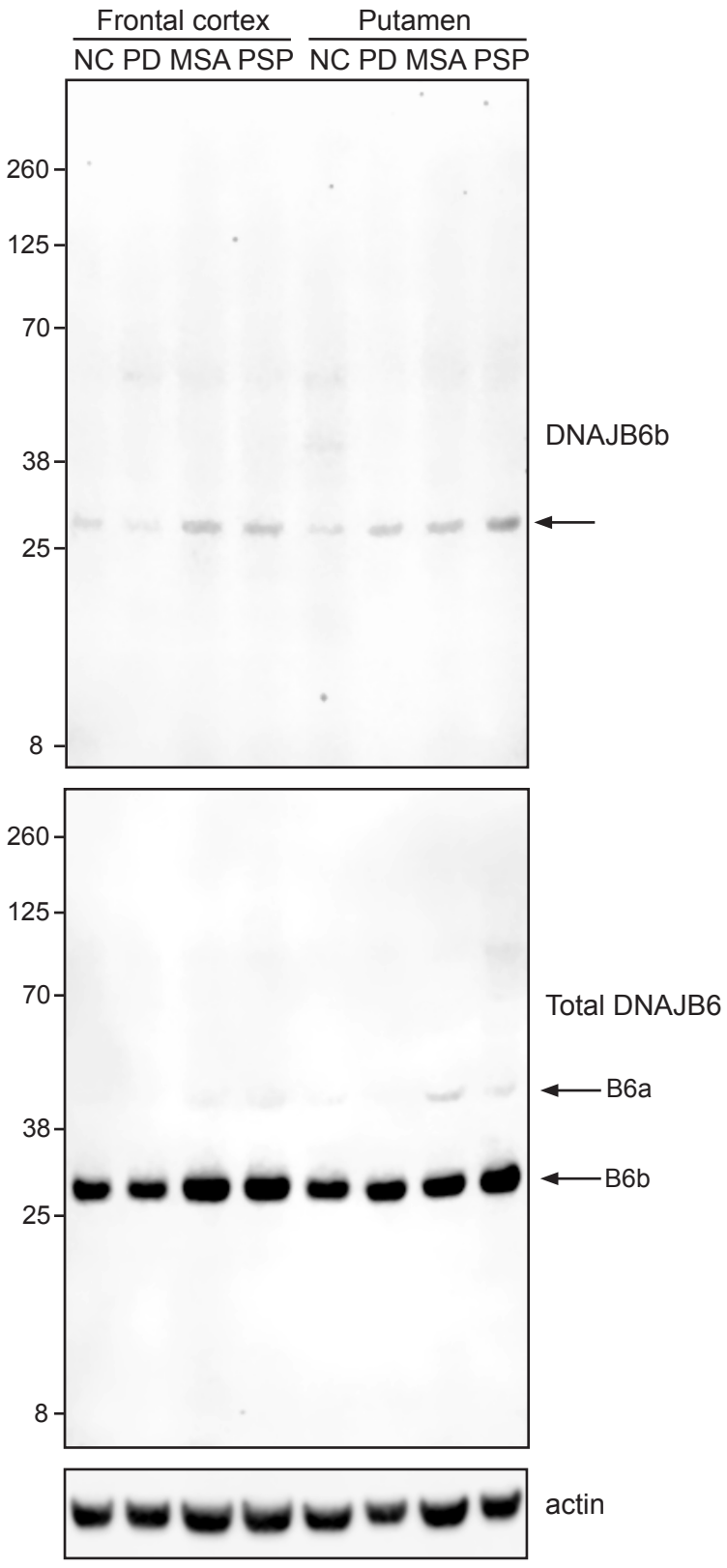
Supplementary Material

DNAJB6 Is Downregulated in Synucleinopathies



Supplementary Figure 1. Standard curves and Spike-recovery test for ELISA measurement. *Standard curves:* ELISA standard curves for **A**) DNA and **B**) DNAJB6b. Detection limit were set at 0.5 ng/mL with above 2 x standard deviation from background PBS+BSA01%. *Spike-and-recovery test:* Duplicates of pooled brain homogenates were spiked with 15 ng/mL **C)** DNAJB6 and **D)** DNAJB6b, respectively. Recovery (%) of spiked DNAJB6 and DNAJB6b was calculated by percentage of amount reduced binding from a non-sample spike (15 ng/mL). The recovery for DNAJB6 was 91.4% and for DNJAB6b 82.4%, indicating a small matrix effect. Matrix effect is higher in DNAJB6b, probably due to the dilution factor for the assay which was 1:1, compared to 1:20 for DNAJB6.



Supplementary Figure 2. Western blot depicting the expression of DNAJB6b and total DNAJB6 in representative samples from human brain tissue. This was analyzed by probing membranes with anti-DNAJB6b, anti-total DNAJB6 and HRP conjugated anti-rabbit antibodies, as well as HRP conjugated anti-actin as a loading control.

A

```
1 mv-dyyevlgvqrhaspedikkayrklalkwhpdknpenkeeaerkfkqvaeayevlsdakkrdiydkygkeglnggggg-shfdspfefgftfrnpddvfreffggrdpfsfdffedpf
2 mv-dyyevlgvqrhaspedikkayrkqalkwhpdknpenkeeaerkfkqvaeayevlsdakkrdiydkygkeglnggggggihfdspfefgftfrnpddvfreffggrdpfsfdff-dpf
3 mv-dyyevlgmqrhaspedikkayrkqalkwhpdknpenkeeaerkfkqvaeayevlsdakkrdiydkyskeglnggggggshfdspfefdftfrnpddvfreffggrdpfsfdffedpf
4 mv-dyyevlgvqrhaspedikkayrklalkwhpdknpenkeeaerkfkqvaeayevlsdakkrdiydkygkeglnggggg-shfdspfefgftfrnpddvfreffggrdpfsfdffedpf
5 mv-dyyevlgvhrhasaedikkayrklalkwhpdknpenkeeaerkfkqvaeayevlsdakkrdiydkygkeglnsggggg-shfdsacgfgftfrnpddvfreffggrdpfsfdffedpf
6 mv-dyyevlgvqkhasaedikkayrklalkwhpdknpenkeeaeqqfkqvaeayevlsdakkrdiydrfgkeglinggggshhdn-pfefgftfrnpddvfreffggrdpfsfdffedpf
7 meedyyhilgvtksaspddikkayrklalkwhpdknpndkeeaekrfkeiseayevlsdenkrrdydrygkqglsnrgghyddeymgg----ftfrnpedvfreffgghdpfadffaddpf

1 edffgnrrgprgs-rsrgtgsffsafsg-fpsfgsgfssfdtgftsfgslghgglt sfsstsf-ggsgmgnfksiststkmvngrkittkrivengqerveveedgqlksltingkeqllrldnk
2 ddffgnrrgprgn-rsrgagsffstfsg-fpsfgsgfpafdtgftpfgslghgglt sfsstsf-ggsgmgnfksiststkivngkkittkrivengqerveveedgqlksltingkehllrldnk
3 ddffgnrrgprgs-rsrgagsffsafsv-fpsfgsgfpafdtgftpfgslghgglt sfssasf-ggsgmgnfksiststkivngkkittkrtvengqerveveedgqlksltingkehllrldnk
4 edffgnrrgprgs-rsrgtgsffsafsg-fpsfgsgfssfdtgftsfgslghgglt sfsstsf-ggsgmgnfksiststkmvngrkittkrivengqerveveedgqlksltingkeqllrldnk
5 edffgnrrgprgs-rnrgtgsffsafsg-fpsfgggfssfdtgftsfgslghgglt sfsstaf-ggggmgnyksiststkvvngrkittkrivengqerveveedgqlksltingkeqllrldnk
6 edffggrrgprgs-rsraggsflsafgg-fpafgnafpsfdtgftsfgslghgglt sfsstsflggsgmgnfksvststkivngrkittkrivengqerveveedgqlrsltingkeqllrldnk
7 egffgghrh-rgmsrsrtagpffpgfspfgpsfsgfdtgfspfgpmgggsfssfsssspf----ggggmrnftsiststkfingkrittkrivengqerveveedgqlksltvngkeqllrldsk
```

Yellow highlight: The amino acid residue is conserved across all 7 species. Underscore: The c-terminal sequence unique to DNAJB6b (not present in DNAJB6a).

B

Human	KEQLLRLDNK
Mouse	KEHLLRLDNK
Rat	KEHLLRLDNK
Gibbon Monkey	KEQLLRLDNK
Blue Whale	KEQLLRLDNK
Chicken	KEQLLRLDNK
Zebra Fish	KEQLLRLDSK

C

1. dnaJ homolog subfamily B member 6 isoform b, Sequence ID: NP_005485.1 (the DNAJB6b protein itself – positive control for the hit)

```
Query  1    KEQLLRLDNK  10     Identities: 10/10 Positives: 10/10
            KEQLLRLDNK
Sbjct  232  KEQLLRLDNK  241
```

2. pre-B-cell leukemia transcription factor 4 .Sequence ID: XP_016882815.1 (identical results for other isoforms of this protein)

```
Query  3    QLLRLDN  9        Identities: 7/10 Positives 7/10
            QLLRLDN
Sbjct  97   QLLRLDN  103
```

3. Transmembrane protein 143. Sequence ID: NP_060743.2 (identical results for other isoforms of this protein)

```
Query  1    KEQLLRL  7        Identities: 7/10 Positives 7/10
            KEQLLRL
Sbjct  79   KEQLLRL  85
```

4. DNA excision repair protein ERCC-6-like 2 Sequence ID: NP_064592.3 (identical results for other isoforms of this protein)

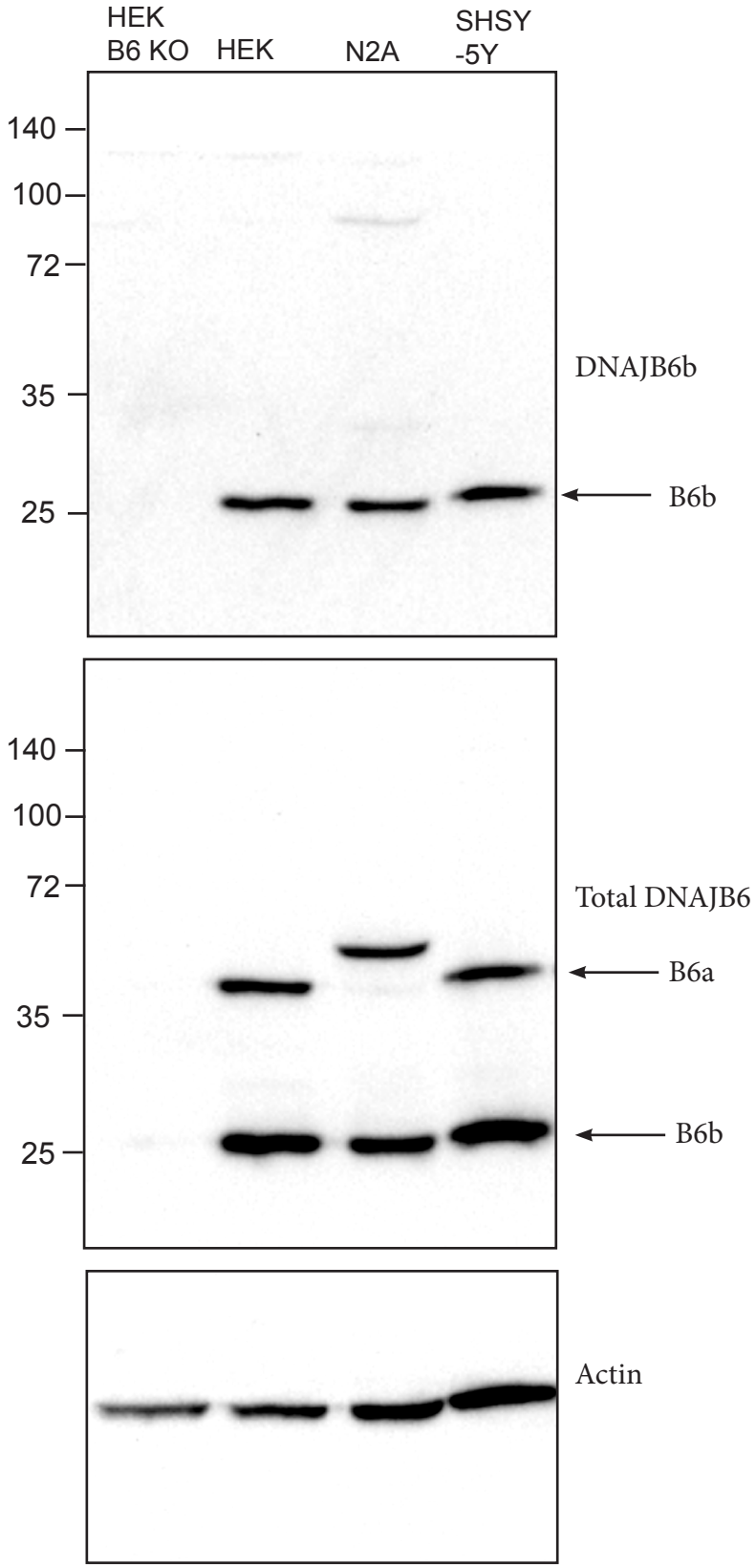
```
Query  1     KEQLLRLDNK  10    Identities: 7/10 Positives 8/10
             KE LL L+NK
Sbjct  1414  KEPLLKLENK  1423
```

5. Neurabin-1 Sequence ID: XP_011514682.1 (identical results for other isoforms of this protein)

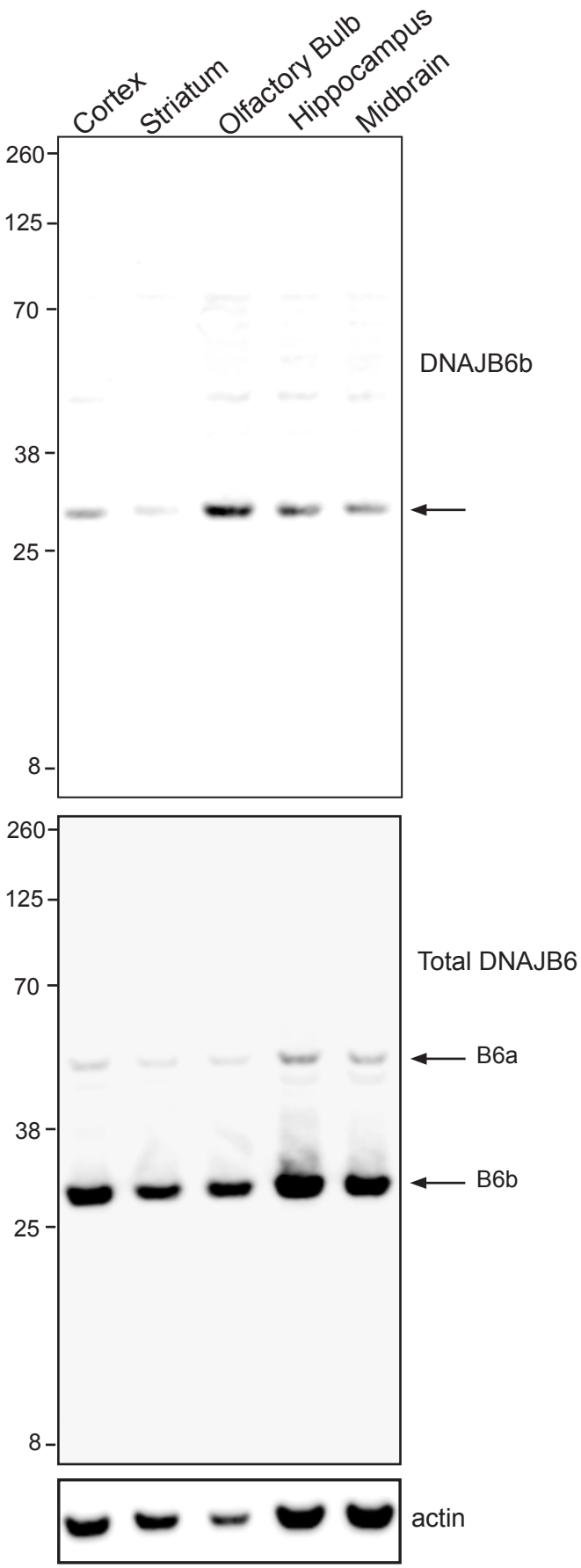
```
Query  2     EQLLRLD-NK  10    Identities: 8/10 Positives 8/10 (gap in the sequence to make the match)

Sbjct  1215  EQLL LD NK. 1224
Sbjct  1314  EQLLQLDGNK  1323
```

Supplementary Figure 3. Sequence analysis of DNAJB6b across species and peptide used for immunization. **A)** comparison of the DNAJB6b sequence between 7 different species: 1. human 2. mouse, 3. rat, 4. gibbon monkey, 5. blue whale, 6. chicken, and 7. zebra fish. **B)** The 10 C-terminal amino acid residues of the same species as shown in (A), enlarged. This human 10aa sequence was used for generating a DNAJB6b specific antibody, by immunization in rabbit. **C)** Searching for peptide sequence KEQLLRLDNK, which was the human peptide sequence on the C-terminus of DNAJB6b used for generating a DNAJB6b specific antibody by immunization in rabbit, using protein blast human database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to analyze for potential redundancy of the 10 amino acid residue peptide sequence unique to DNAJB6b. The result of the positive control and the 4 nearest matching hits in humans are displayed.



Supplementary Figure 4. Western blot is the same is shown as shown in Figure 1B, depicting the expression of DNAJB6b and total DNAJB6 in selected cell lines, but here we present the full blot when stained with anti-DNAJB6b or anti-total DNAJB6 (top and middle blots, respectively).



Supplementary Figure 5. Western blot is the same is shown as shown in Figure 3C, depicting the expression of DNAJB6b and total DNAJB6 in selected brain tissues, but here we present the full blot when stained with anti-DNAJB6b or anti-total DNAJB6 (top and middle blots respectively).