

Expanded View Figures

Figure EV1. Optimising the conditions used for ABOLISH.

- A Schematic of the original growth conditions used to test Bpl1-AID*-9myc degradation and endogenous biotinylation reduction.
- B Anti-Myc and streptavidin blots of cells expressing Bpl1-AID*-9myc and OstTIR1 grown overnight and back-diluted for ~ 4 h in regular synthetic (SD) or reduced biotin (RB) media. To the cells grown in RB media, auxin was either omitted (–) or added 15 min, 30 min, 1 h or 2 h prior to harvesting. Similarly, biotin was either omitted (–) or added 5 min prior to harvesting. Band intensities for the most prominently endogenously biotinylated proteins in lanes 1, 2, 3 and 7 were quantified using Fiji software.
- C Anti-HA blots confirming the expression of BioID2/TurboID-HA-tagged Emc6 and Sbh1. An anti-Myc blot was included for the strains containing the ABOLISH system.
- D Western blot analysis of cells expressing either TurboID-HA-Emc6 or TurboID-HA-Sbh1 together with the ABOLISH system. Cells were grown in SD or RB media as in (B). Auxin and biotin were either omitted (–) or added (+) 1.5 h and 30 min, respectively, before harvesting.
- E Western blot analysis of cells expressing TurboID-HA-Emc6 and the ABOLISH system. Cells were grown in several different conditions: (i) overnight and back-diluted in rich media (YPD) containing auxin; (ii) overnight and back-diluted in regular SD containing auxin; (iii) overnight and back-diluted in RB media with auxin and biotin either omitted (–), added for 1.5 h and 30 min, respectively, before harvesting (+), or added for the entire duration of the back-dilution (++); (iv) overnight and back-diluted in regular SD with auxin and biotin treatments as in (iii). Sec61 was used as an untagged ER membrane protein control.

Data information: For panels B–E, H3 (histone H3) or Actin was used as loading controls. Back-dilution times were all ~ 4 h.

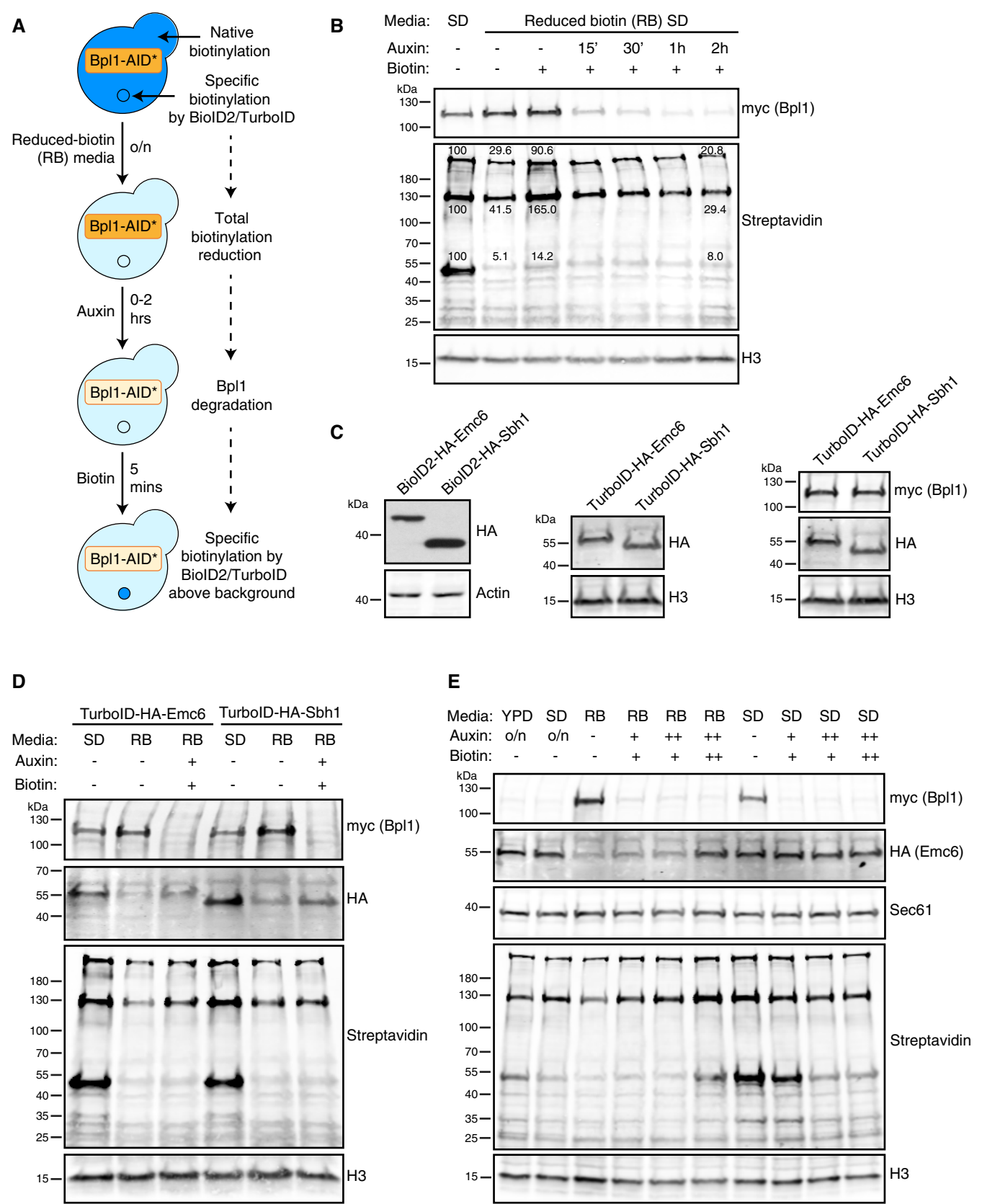


Figure EV1.

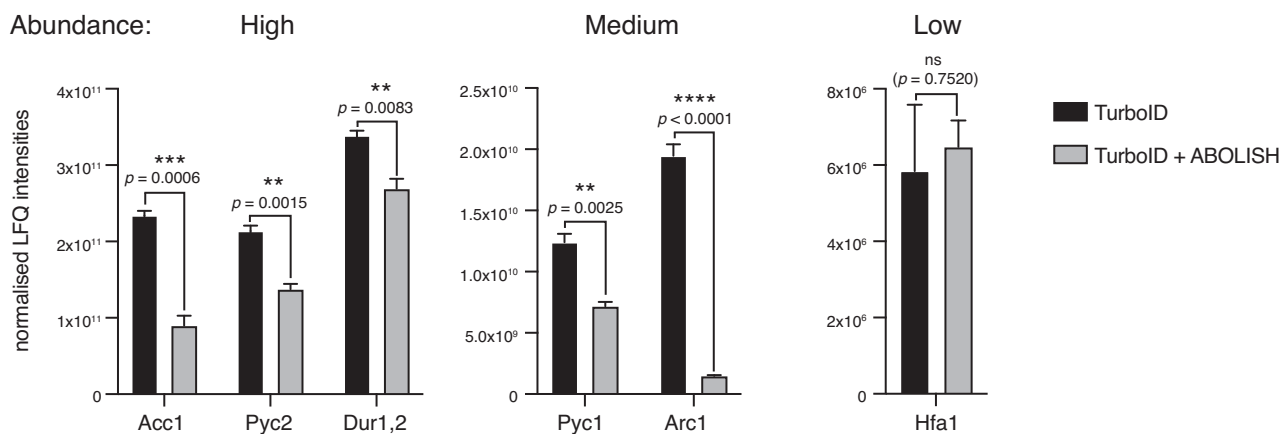


Figure EV2. Quantitation of ABOLISH in LC-MS/MS.

Bar graphs showing the normalised label-free quantification (LFQ) intensities of endogenously biotinylated proteins, with and without ABOLISH, as measured by LC-MS/MS from biological triplicates. Proteins are grouped into either high, medium or low abundance to enable clear visualisation of the differences. Shown are the standard error of the mean (SEM) and *P*-values from two-tailed unpaired *t*-tests demonstrating significance or not (ns).

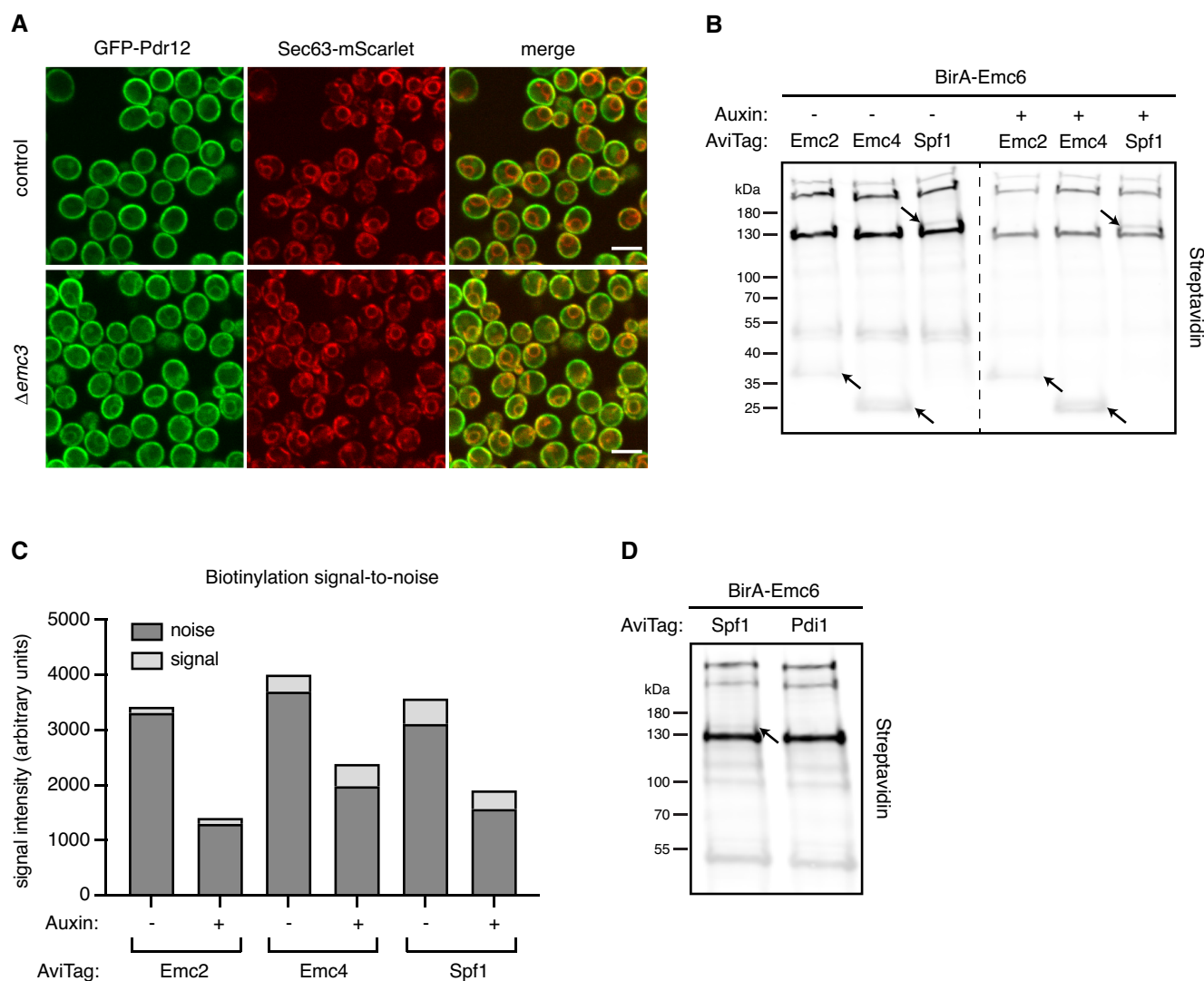


Figure EV3. Contribution of the ABOLISH system to noise reduction in BirA-AviTag blotting.

- A Fluorescence microscopy images of control and $\Delta emc3$ strains containing Pdr12 N' tagged with GFP and Sec63 C' tagged with mScarlet. Sec63 localisation was unchanged upon loss of Emc3 and no significant difference was found in signal intensity ($P = 0.1156$). Scale bar = 5 μm .
- B Streptavidin blot of diploid strains expressing either AviTag-Emc2, -Emc4, or -Spf1 together with BirA-Emc6, grown in media with or without auxin. 15 μg of whole-cell lysate from each sample was loaded onto the gel. The arrows indicate the bands corresponding to the molecular weight of each of the AviTagged proteins.
- C Quantitation of the streptavidin signal from endogenously biotinylated proteins (noise) and biotinylated AviTagged proteins (signal) show a reduction in the background noise by $\sim$ half when ABOLISH is activated.
- D Streptavidin blot of diploid strains expressing either AviTag-Spf1 or -Pdi1 together with BirA-Emc6, grown in media containing auxin. The arrow indicates the band corresponding to the molecular weight of the AviTagged protein.

Figure EV4. Quality control checks on biotinylation toolkit libraries.

- A–C Western blot analysis for selected strains from the BioID2-HA (A) TurboID-HA (B) and TurboID-HA/ABOLISH (C) libraries. For the BioID2-HA library, Phm6, Din7, Skt5, Rcr1 and Mig2 all have a low endogenous expression (relative intensity values of ≤ 27 , Weill et al, 2018), which likely explains why they were not readily detectable. 'Control (28)' refers to BioID2-HA not tagged to any protein, and 'WT' denotes lysate ran from the BY4741 laboratory strain to highlight nonspecific bands; the most prominent of which are marked with an asterisk. For all panels, H3 (histone H3) is used as a loading control. The expected molecular weight in kDa for each tested protein including their tag is written in parentheses after the protein name.

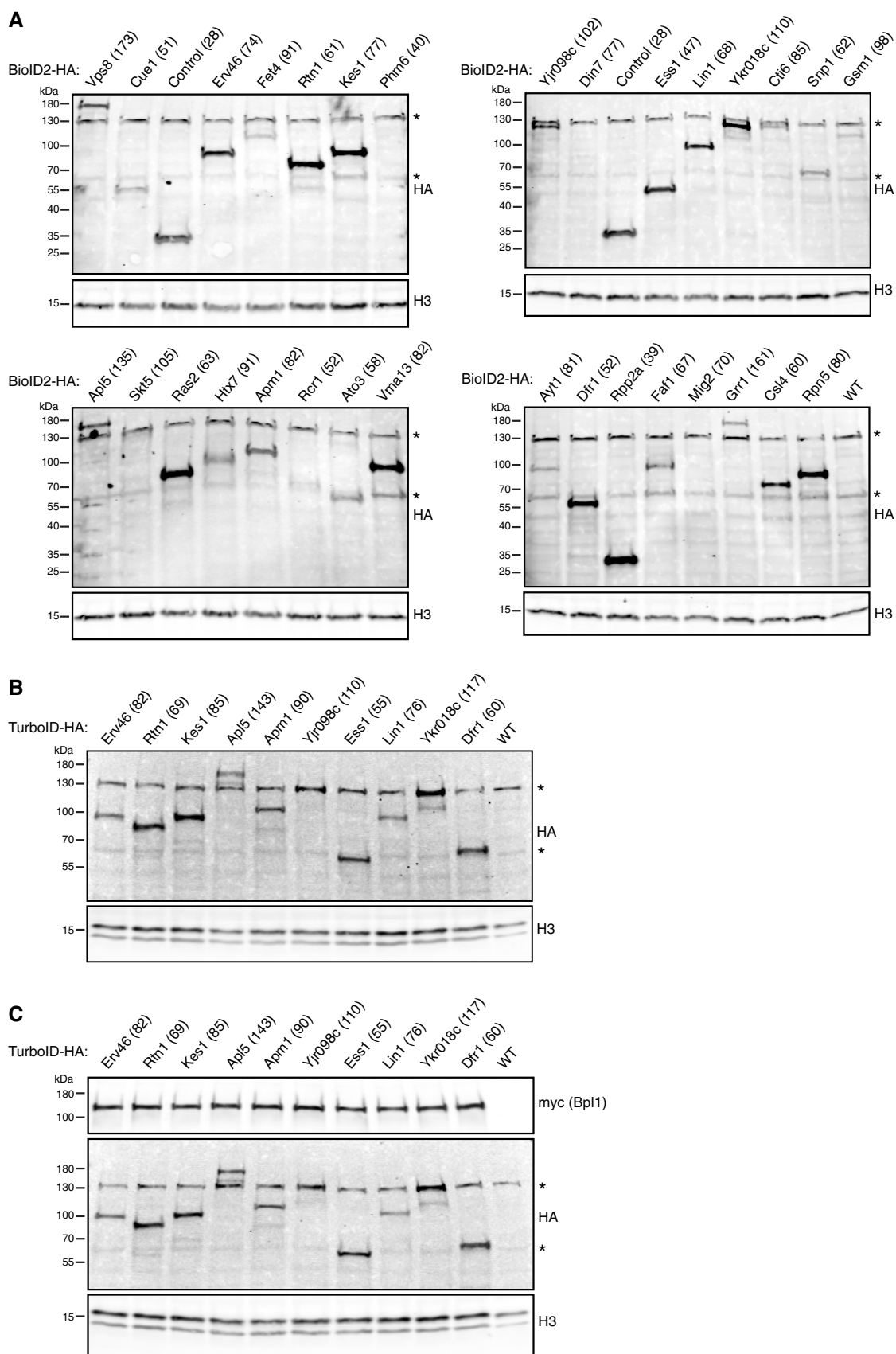


Figure EV4.