Supplementary Data

Latrepirdine (Dimebon™) Enhances Autophagy and Reduces Intracellular GFP-Αβ42 Levels in Yeast

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Supplementary Figure 1. Aggregation states of GFP-Ab fusion proteins. A) Yeast cells expressing GFP-Ab$_{42}$ and GFP-Ab$_{38}$ (19:34) were lysed using glass bead homogenization in 1X PBS only or containing 1% SDS or non-ionic detergents, 1% TritonX100 (TX100) and 1% dodecylmalto side (DDM). 50µg of total protein extract was loaded on 4–12% Bis-Tris gels (MES buffering) and analyzed by immunoblotting (anti-Ab, W02). Abundant levels of GFP-Ab$_{42}$ (19:34) was present in the PBS/bead homogenization extract, 1% SDS, 1% TX100, and 1% DDM extract (lanes 2, 4, 6, and 8 respectively). However, GFP-Ab$_{38}$ was found largely associated in the 1% SDS extract (lane 3) whereas low levels were detected in PBS/bead homogenization, 1% TX100, and 1% DDM extracts (lanes 1, 5 and 7).

B) To determine the native aggregation states of the GFP-Ab fusion proteins, the yeast protein extracts (50µg) were analyzed by Blue Native-PAGE using 4–12% Bis-Tris gels. A smear ranging from ~43 kDa and lower representing monomeric GFP-Ab was prominent in the extracts from yeast cells expressing GFP-Ab$_{42}$ or GFP-Ab$_{38}$ (19:34) (lanes 1–8). In 1% TX100 or 1% DDM extracts, abundant monomeric GFP-Ab$_{38}$ (19:34) was present in the transformant yeast cells (lanes 6 and 8). Very low levels of the monomers were present in 1% TX100 and 1% DDM extracts from cells expressing GFP-Ab$_{42}$ (lanes 5 and 7); instead larger molecular weight protein aggregates were the prominent species. No such aggregate protein species was found as GFP-Ab$_{42}$/PBS (lane 1), GFP-Ab$_{38}$/1% SDS extract (lane 3), or GFP-Ab$_{38}$ (19:34) extracts (lanes 2, 4, 6, 8).