Assembly-induced folding regulates interleukin 12 biogenesis and secretion

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Running title: *molecular mechanism of IL12 biogenesis*

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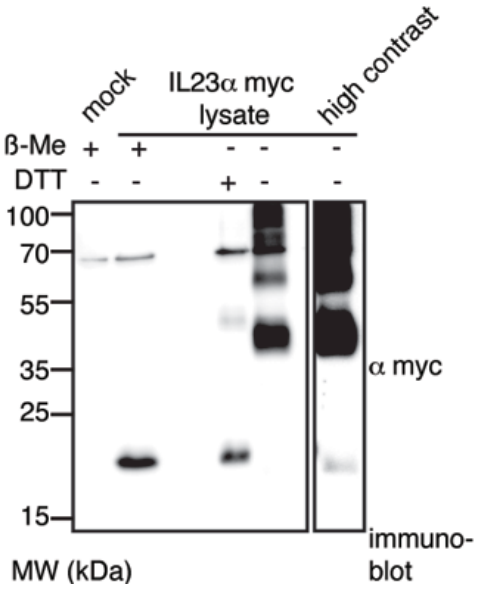
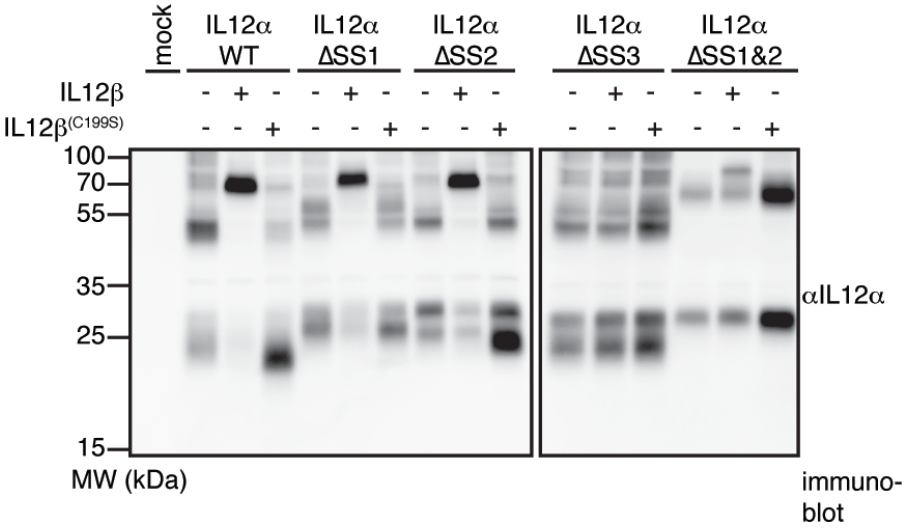
- Supplemental Data -

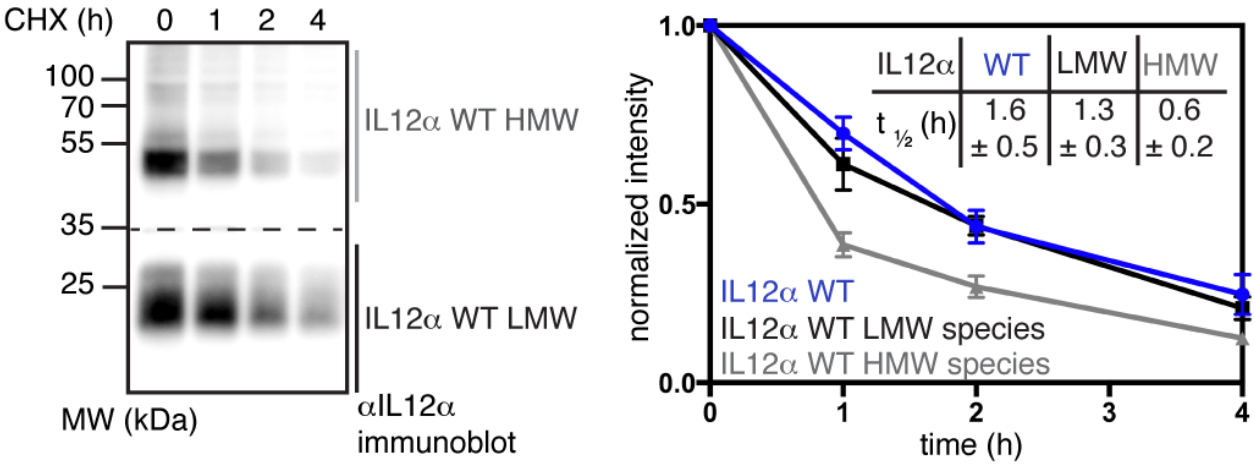
Supplementary Figures



Supplemental Figure 1. Isolated IL23α shows comparable redox species to isolated IL12α. The oxidation state of IL23α with a C-terminal Myc-tag was analyzed by non-reducing SDS-PAGE. Where indicated, samples were treated with β-mercaptoethanol (β-Me) after cell lysis to provide a size standard for completely reduced proteins or cells were treated with DTT for 1 h before lysis to reduce disulfide bonds in the cell. 2% lysate was applied to the gel and blotted with anti-Myc antibody. To make the monomeric IL23α visible, a high contrast image is shown on the right.



Supplemental Figure 2. Influence of IL12α disulfide bridges on IL12α redox status in the presence of IL12β. The redox status of IL12α alone and in the presence of IL12β or IL12β^{C199S} was analyzed by non-reducing SDS-PAGE. Samples were treated with brefeldin A for 1 h before lysis to inhibit protein secretion. 293T cells were co-transfected with the indicated IL12α-/β-subunits and 2% lysate was applied to the gel and blotted with anti-IL12α antibody.



Supplemental Figure 3. Measurement of IL12α redox species turnover by cycloheximide (CHX) chase assays. For CHX chases 293T cells were transfected with IL12α WT and incubated with CHX for up to 4 h. Cell lysates were analyzed under non-reducing conditions by immunoblotting with anti-IL12α antibody. The anti-IL12α immunoblot signal was normalized to the signal of high molecular weight (HMW) (larger than 35 kDa) and low molecular weight (LMW) species (smaller than 35 kDa) which were present at the start of the chase. Half-lifes of LMW (■) and HMW (▲) species were compared with IL12α WT (●) from Fig 4A, (n=4±SEM). Half-lives from exponential fits of the curves (±SD) are shown in the table.