## Supplemental:

# Cross-seeding of wild-type Amyloid-β with Arctic but not Italian familial mutants accelerates fibril formation in Alzheimer's Disease

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## Supplemental Figures S1 –S12

**Supplemental Figure S1:** TEM images at equilibrium phase Arctic and Italian Isoforms.

**Supplemental Figure S2:** Aβ42(Italian) monomer cross-seeding with Aβ(WideType) and Aβ(Italian) fibrils.

**Supplemental Figure S3**: Aβ40(Arctic) monomer cross-seeding with Aβ(WideType) and Aβ(Arctic) fibrils.

**Supplemental Figure S4:** Aβ40(WildType) monomer cross-seeding with Aβ(WildType), Aβ(Italian) and Aβ(Arctic) fibrils.

**Supplemental Figure S5:** Aβ42(WildType) monomer cross-seeding with Aβ(Arctic), Aβ42(Italian) and Aβ(WildType) fibrils.

**Supplemental Figure S6:** Tabulation of mean half-times for cross-seeding conditions.

**Supplemental Figure S7:** Cross-seeding of WildType Aβ40 fibrillization with seeds at different concentrations.

**Supplemental Figure S8**: C-Amidated Aβ42

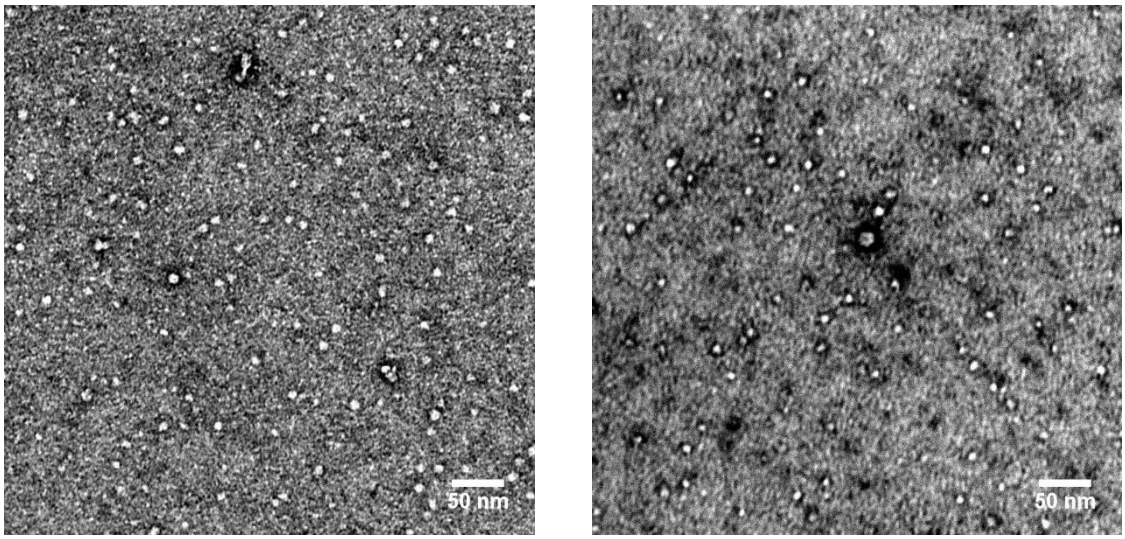
**Supplemental Figure S9:** TEM images of both seeded and unseeded Aβ42(WildType) and Aβ40(Arctic).

**Supplemental Figure S10:** TEM images of both seeded and unseeded Aβ40(Arctic).

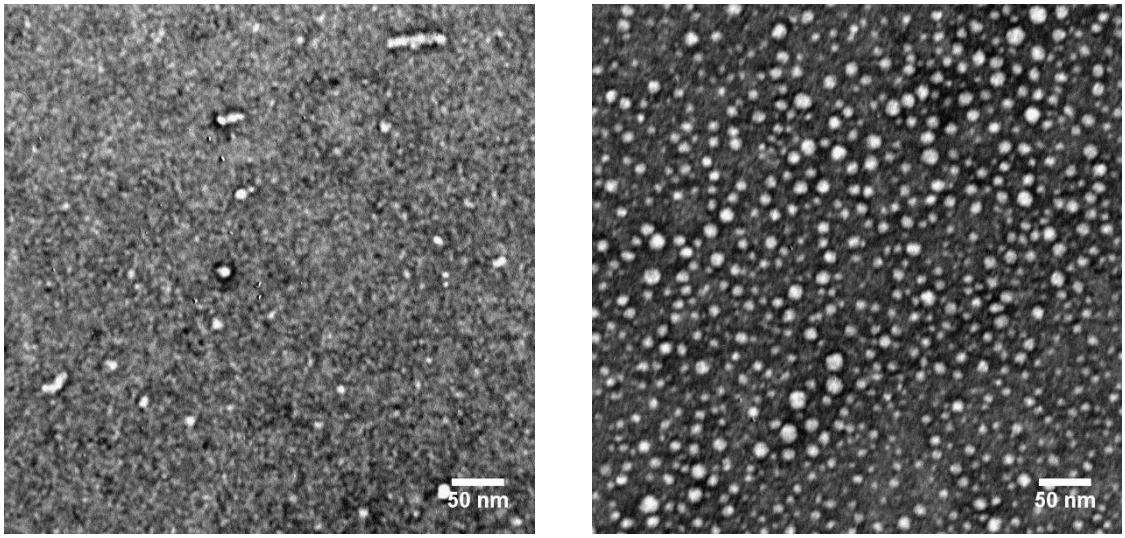
**Supplemental Figure S11:** TEM images of both seeded and unseeded Aβ40(Arctic).

**Supplemental Figure S12:** TEM images of both seeded and unseeded Aβ40(WildType).

## a) Aβ40(Arctic)

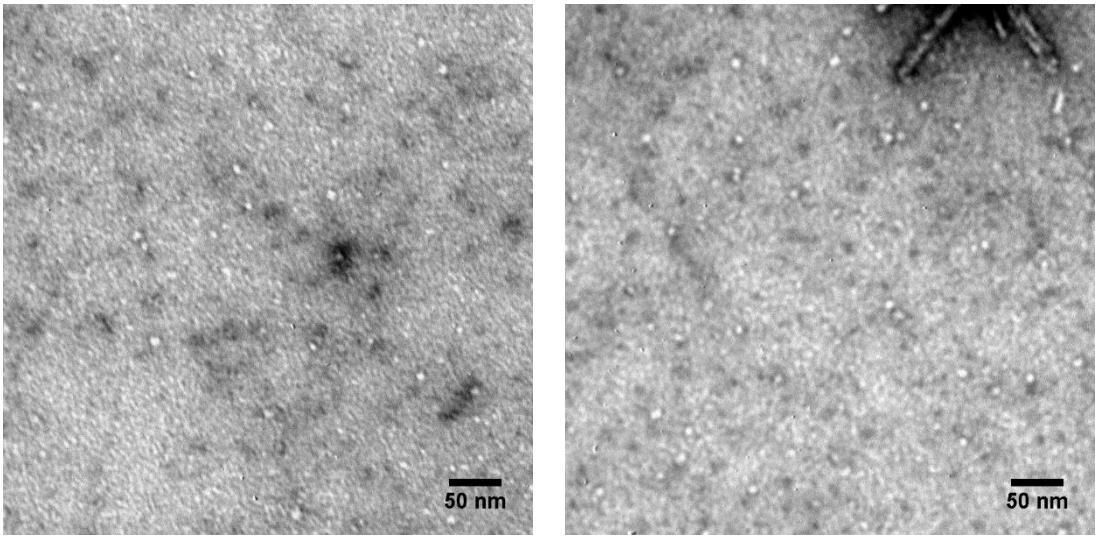



## b) Aβ42(Arctic)

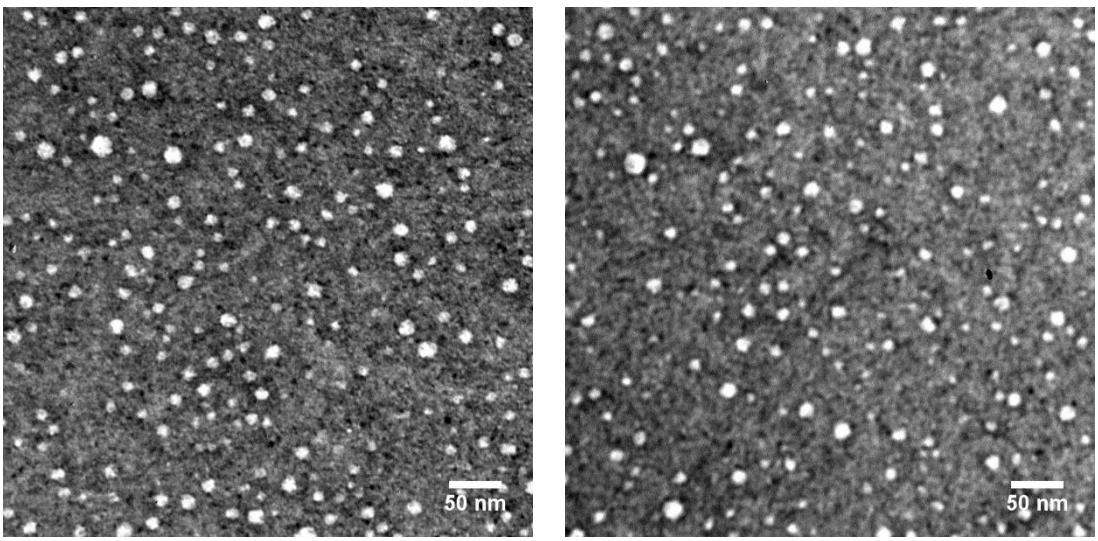



**Supplemental Figure S1: TEM images at equilibrium phase Arctic and Italian Isoforms.** Many oligomers as well as fibrils are observed at equilibrium for four mutant Aβ isoforms: a) Aβ40(Arctic); b) Aβ42(Arctic); c) Aβ40(Italian); d) Aβ42(Italian). Negatively stained TEM images generated from 10 μM Aβ isoforms. Once ThT has reached maximum after 50 hrs incubation at pH 7.4, HEPES buffer (30 mM) and NaCl (160 mM).

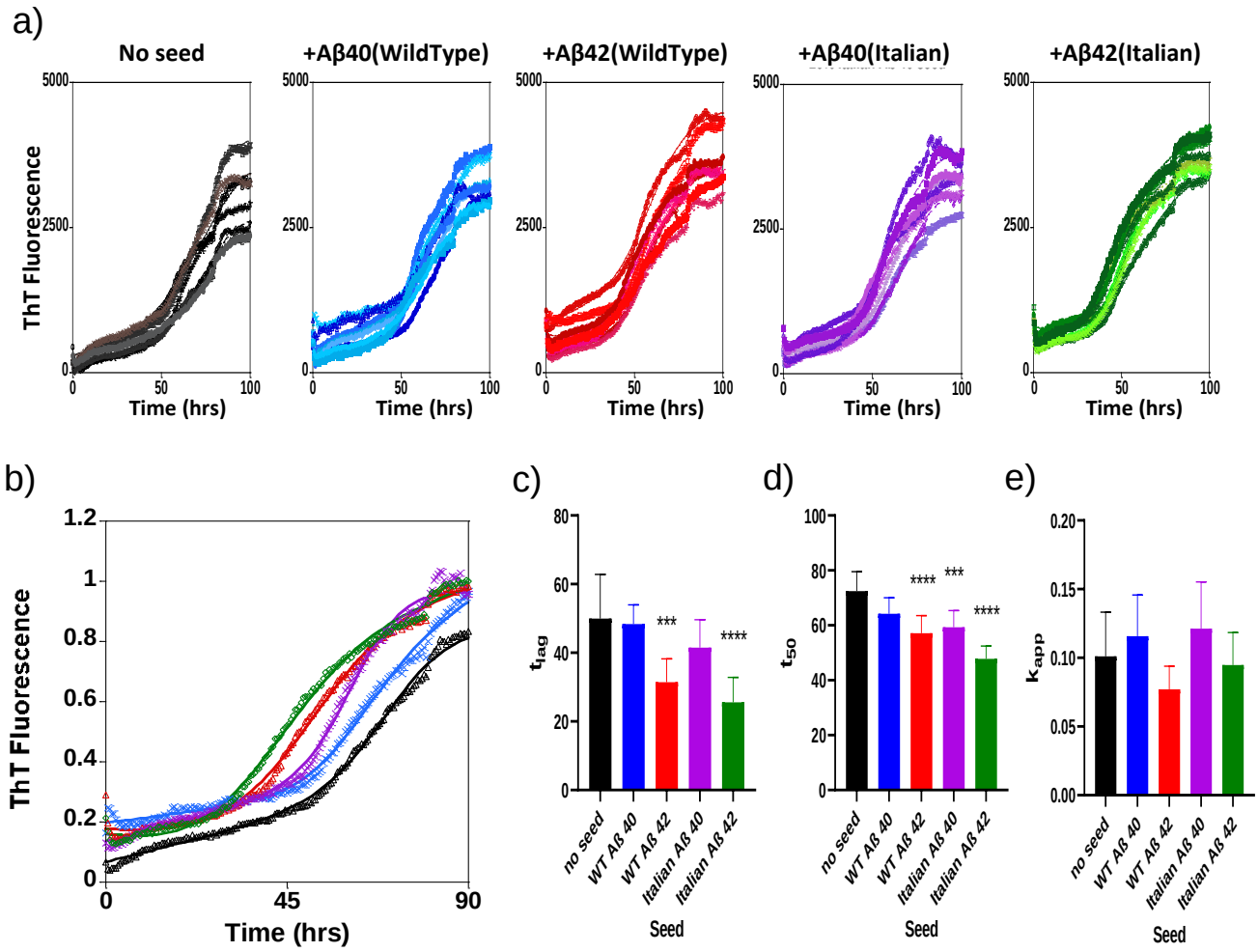
## c) Aβ40(Italian)



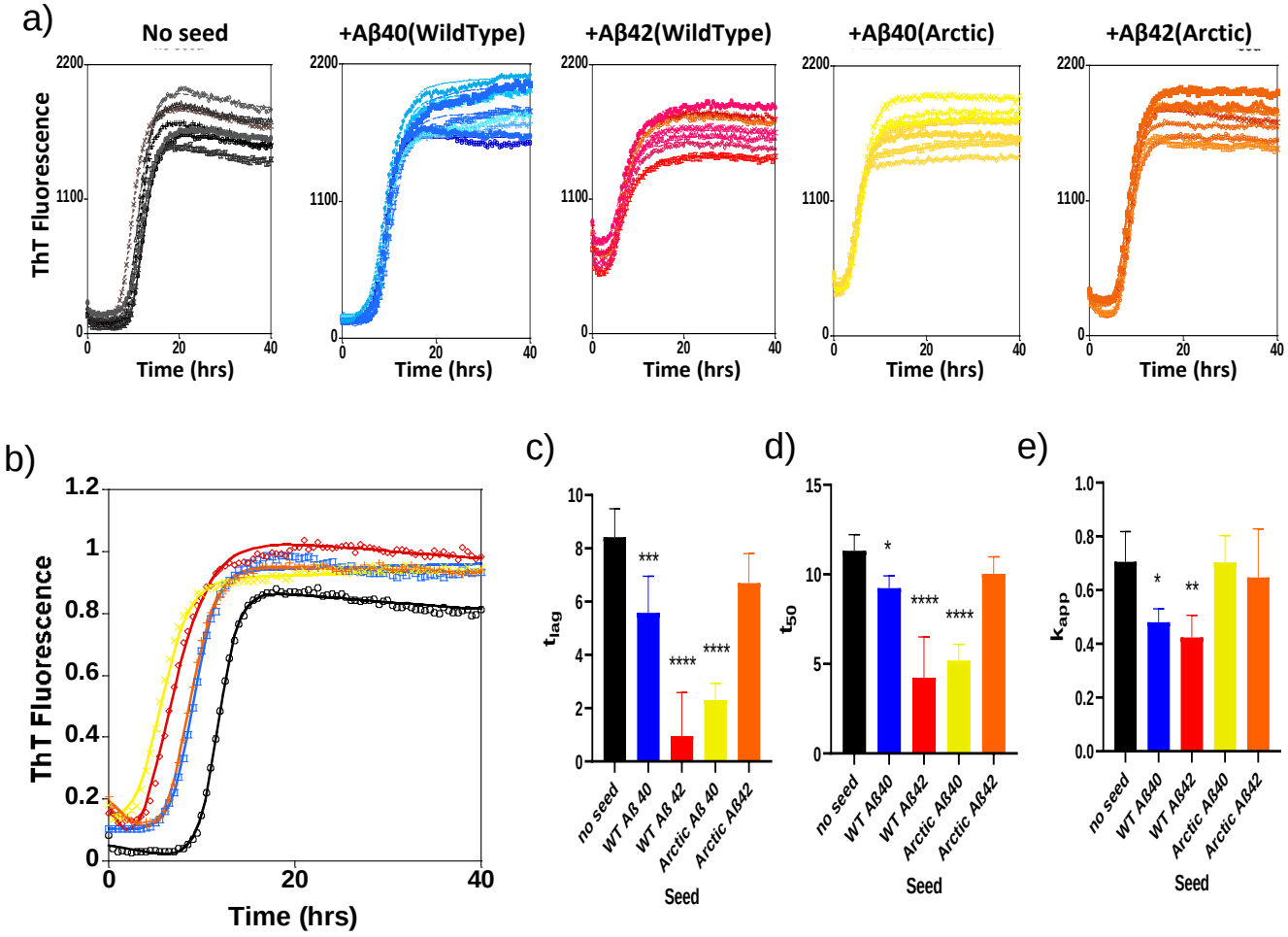

## d) Aβ42(Italian)



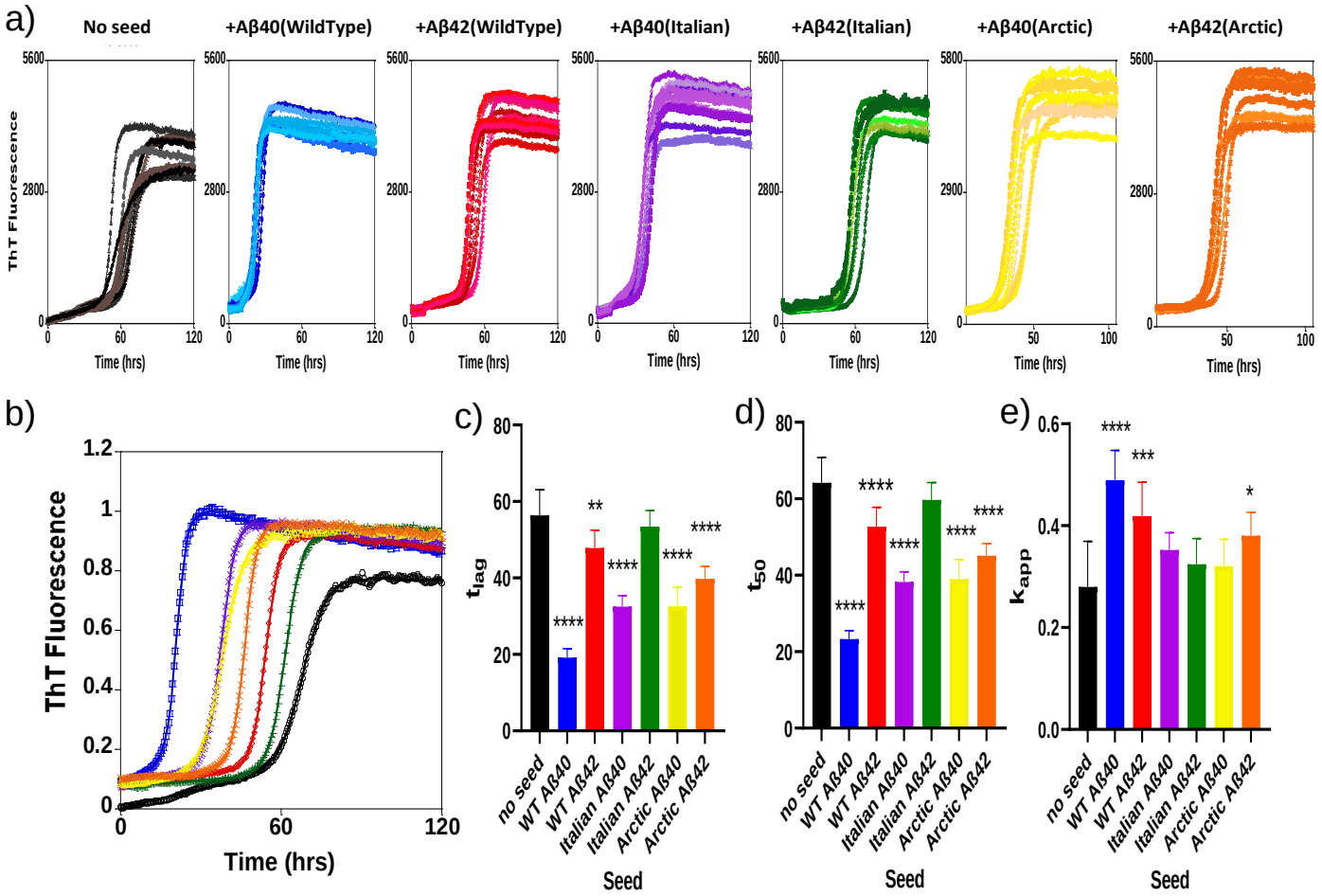

**Supplemental Figure S1: TEM images at equilibrium phase Arctic and Italian Isoforms.** Many oligomers as well as fibrils are observed at equilibrium for four mutant Aβ isoforms: a) Aβ40(Arctic); b) Aβ42(Arctic); c) Aβ40(Italian); d) Aβ42(Italian). Negatively stained TEM images generated from 10 μM Aβ isoforms. Once ThT has reached maximum after 50 hrs incubation at pH 7.4, HEPES buffer (30 mM) and NaCl (160 mM).



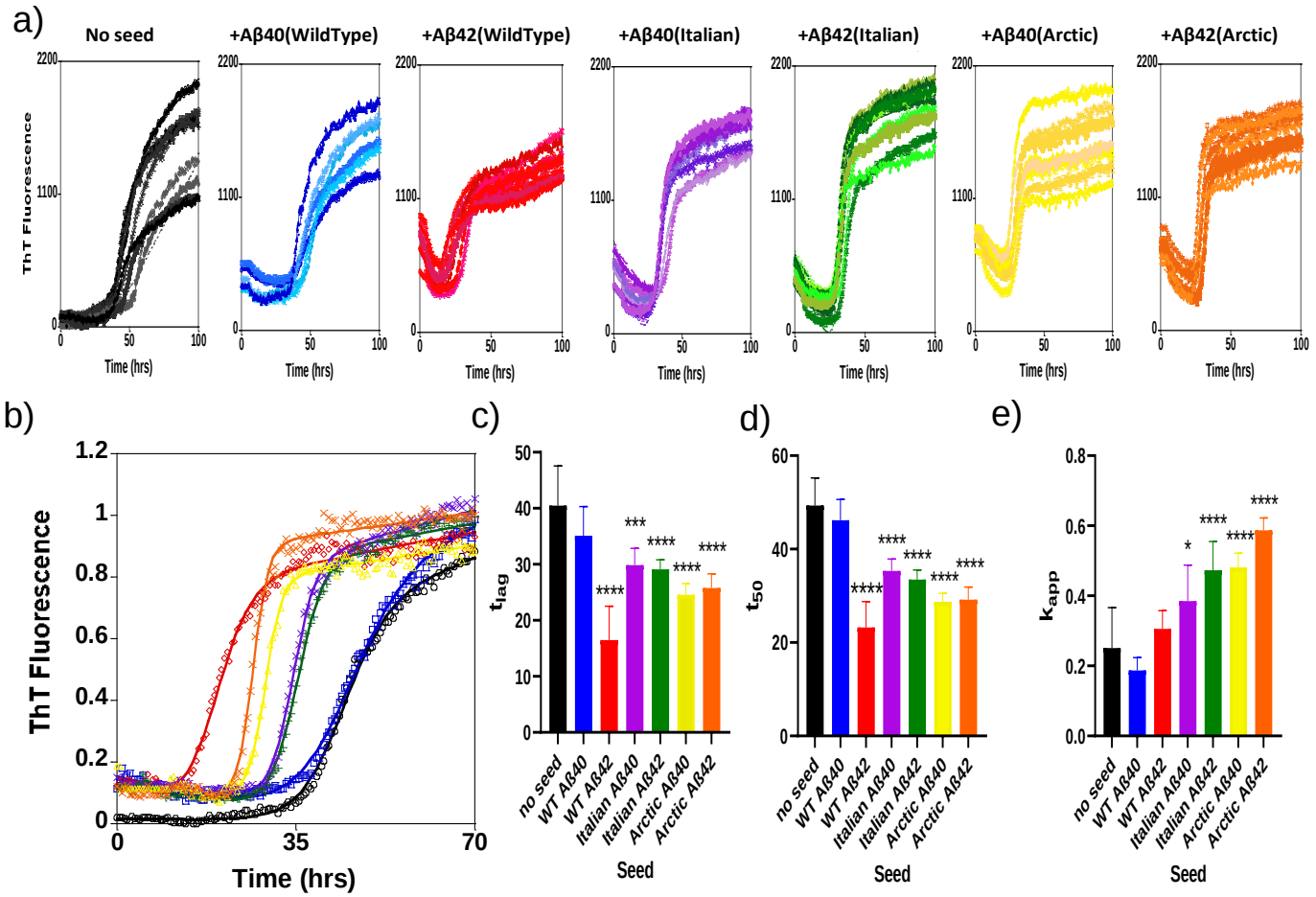

**Supplemental Figure S2: Aβ42(Italian) monomer cross-seeding with Aβ(WideType) and Aβ(Italian) fibrils.** a) Fibril formation of monomeric Aβ42(Italian) in presence of a range Aβ isoform fibril seeds (10 % w/w): No seed (black); Aβ40(WildType) (blue); Aβ42(WildType) (red); Aβ40(Italian) (purple) and; Aβ42(Italian) (green). b) Typical representative (median) single trace of Aβ42(Italian) in the absence and presence of different seeds, same colours. Empirical kinetic parameters for: $t_{lag}$ (c); $t_{50}$ (d) and $k_{app}$ (e) of Aβ42(Italian) fibril formation, mean from n=9 for each condition, error bars are standard deviation. Total Aβ is 10 μM at pH 7.4. Self-seeding with Aβ42(Italian) fibrils (in green) most effectively nucleates fibril formation, with a large reduction in the lag-time. One-way ANOVA test, a comparison between unseeded and seeded kinetics ****P ≤ 0.0001, ***P ≤ 0.001.




**Supplemental Figure S3: Aβ40(Arctic) monomer cross-seeding with Aβ(WideType) and Aβ(Arctic) fibrils.** a) Fibril formation of monomeric Aβ40(Arctic) in presence of a range Aβ isoform fibril seeds (10 % w/w): No seed (black); Aβ40(WildType) (blue); Aβ42(WildType) (red); Aβ40(Arctic) (yellow) and; Aβ42(Italian) (orange). b) Typical representative (median) single trace of Aβ40(Arctic) in the absence and presence of different seeds, same colours. Empirical kinetic parameters: $t_{lag}$ (c), $t_{50}$ (d) and $k_{app}$ (e) of Aβ40(Arctic) fibril formation, mean from n=9 for each condition, error bars are standard deviation. Total Aβ is 10 μM at pH 7.4. Self-seeding with Aβ40(Arctic) fibrils (in yellow) effectively nucleates fibril formation, surprisingly Aβ42(WildType) (in red) is also very effective at nucleating fibril formation, with a large reduction in the lag-time. One-way ANOVA test, a comparison between unseeded and seeded kinetics , *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.



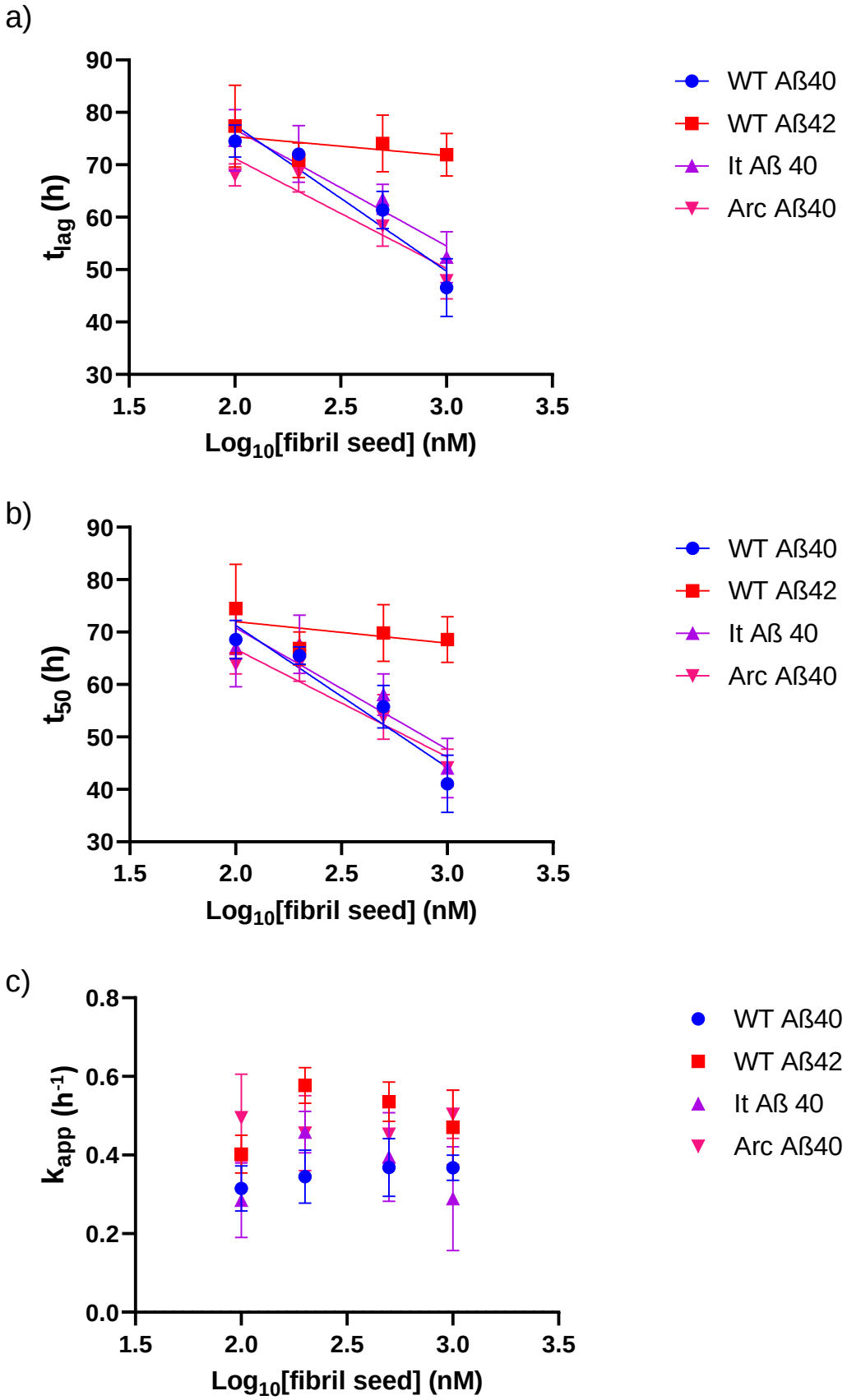

**Supplemental Figure S4: Aβ40(WildType) monomer cross-seeding with Aβ(WildType), Aβ(Italian) and Aβ(Arctic) fibrils.** a) Fibril formation of monomeric Aβ40(WildType) in presence of a range Aβ isoform fibril seeds (10 % w/w): no seed (black); Aβ40(WildType) (blue); Aβ42(WildType) (red); Aβ40(Italian) (purple); Aβ42(Italian) (green); Aβ40(Arctic) (yellow) and; Aβ42(Arctic) (orange); b) Typical representative (median) single trace of Aβ40(WildType) in the absence and presence of different seeds. Empirical kinetic parameters: $t_{lag}$ (c), $t_{50}$ (d) and $k_{app}$ (e) of Aβ40(WildType) fibril nucleation, mean from n=9 for each condition, error bars are standard deviation. Total Aβ is 10 μM at pH 7.4. Self-seeding with Aβ40(WildType) fibrils (in blue) effectively nucleates fibril formation, with a large reduction in the lag-time. One-way ANOVA test, a comparison between unseeded and seeded kinetics: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.



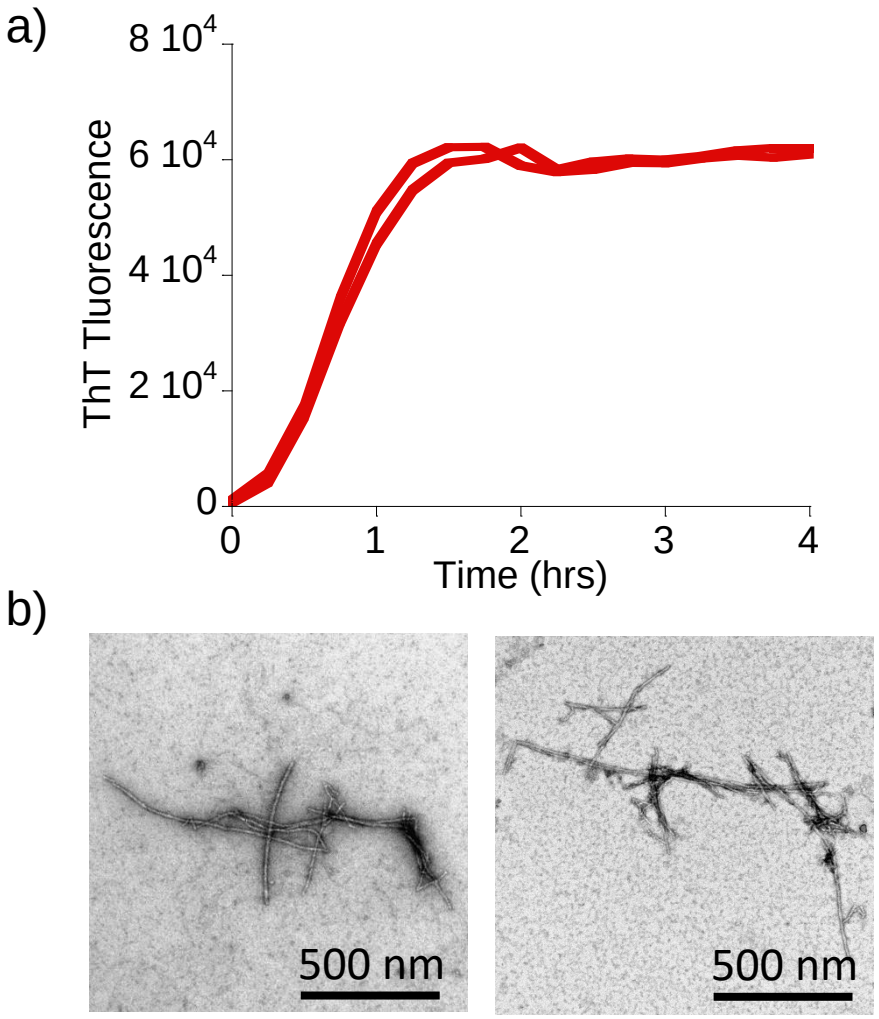

**Supplemental Figure S5: Aβ42(WildType) monomer cross-seeding with Aβ(Arctic), Aβ42(Italian) and Aβ(WildType) fibrils.** a) Fibril formation of monomeric Aβ42(WildType) in presence of a range Aβ isoform fibril seeds (10 % w/w): No seed (black); Aβ40(WildType) (blue); Aβ42(WildType) (red); Aβ40(Italian) (purple); Aβ42(Italian) (green); Aβ40(Arctic) (yellow) and; Aβ42(Italian) (orange). b) Typical representative (median) single trace of Aβ42(WildType) in the absence and presence of different seeds, same colours. Empirical kinetic parameters: $t_{lag}$ (c), $t_{50}$ (d) and $k_{app}$ (e) of Aβ42(WildType) fibril formation, mean from n=9 for each condition, error bars are standard deviation. Total Aβ is 10 μM at pH 7.4. Self-seeding with Aβ42(WildType) fibrils (in red) effectively nucleates fibril formation as do both Arctic mutants, with a large reduction in the lag-time. One-way ANOVA test, a comparison between unseeded and seeded kinetics *P ≤ 0.05, ***P ≤ 0.001, ****P ≤ 0.0001.

| $t_{50}$(%) | | No seed | + seeds of: Wt40 | Wt42 | It40 | It42 | Arc40 | Arc42 |
|---|---|---|---|---|---|---|---|---|
| Monomers of | **Wt40** | 100% | 36% | 82% | 60% | 93% | 61% | 70% |
| | **Wt42** | 100% | 93% | 47% | 72% | 68% | 58% | 59% |
| | **It40** | 100% | 61% | 79% | 34% | 72% | — | — |
| | **It42** | 100% | 87% | 79% | 82% | 66% | — | — |
| | **Arc40** | 100% | 82% | 37% | — | — | 46% | 87% |

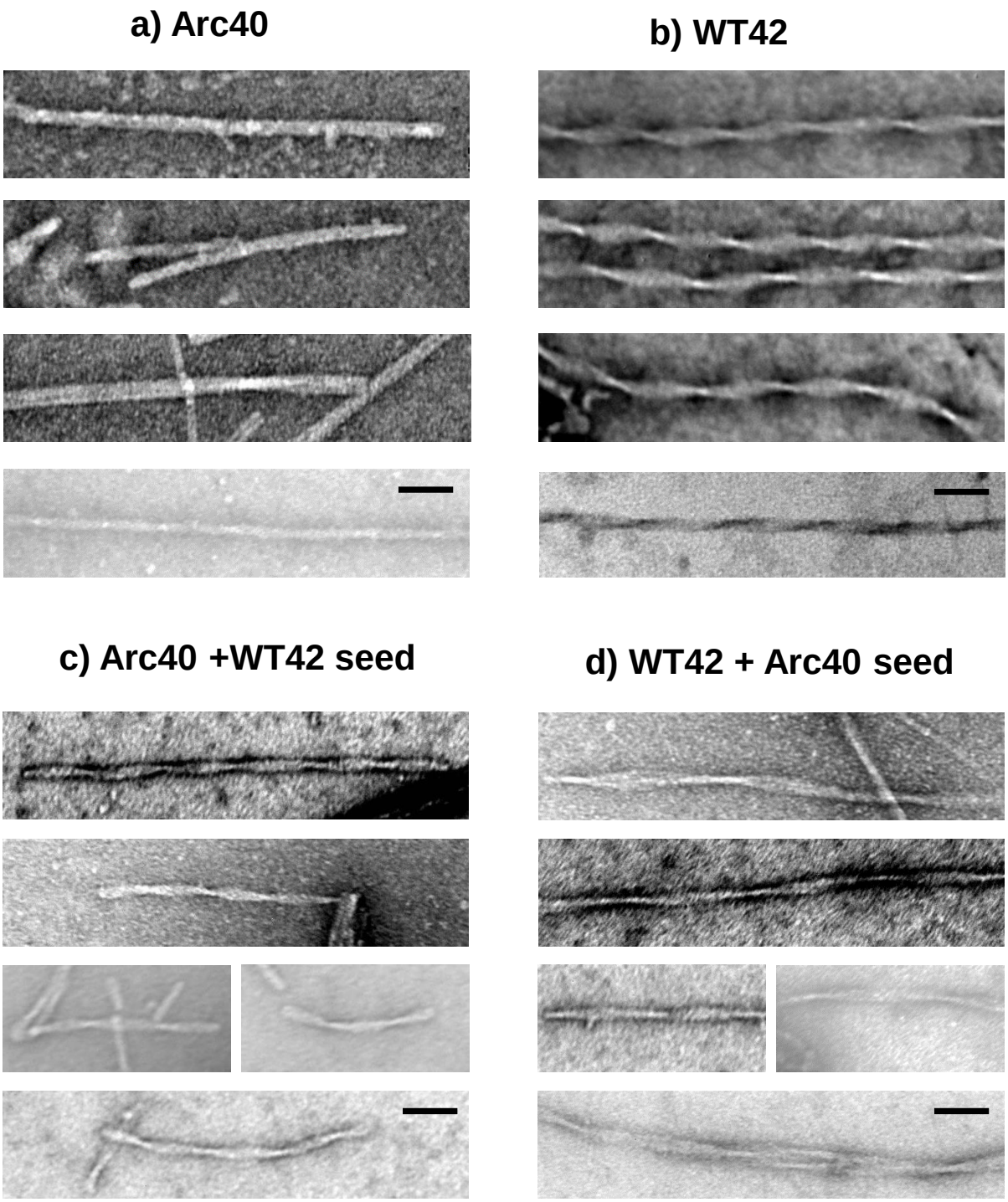
**Supplemental Figure S6: Tabulation of mean half-times for cross-seeding conditions**. a) summary of half-time values for cross-seeding conditions in hours b) summary of half-times for cross-seeding conditions relative to non-seeded monomer. $\mathbf{t}_{50}$ presented as a percentage, relative to non-seeded monomer (100%). Red highlighting strong seeding, orange indicates some seeding, and green, minimal seeding. Wild-Type (Wt), Italian (It) and Arctic (Arc).



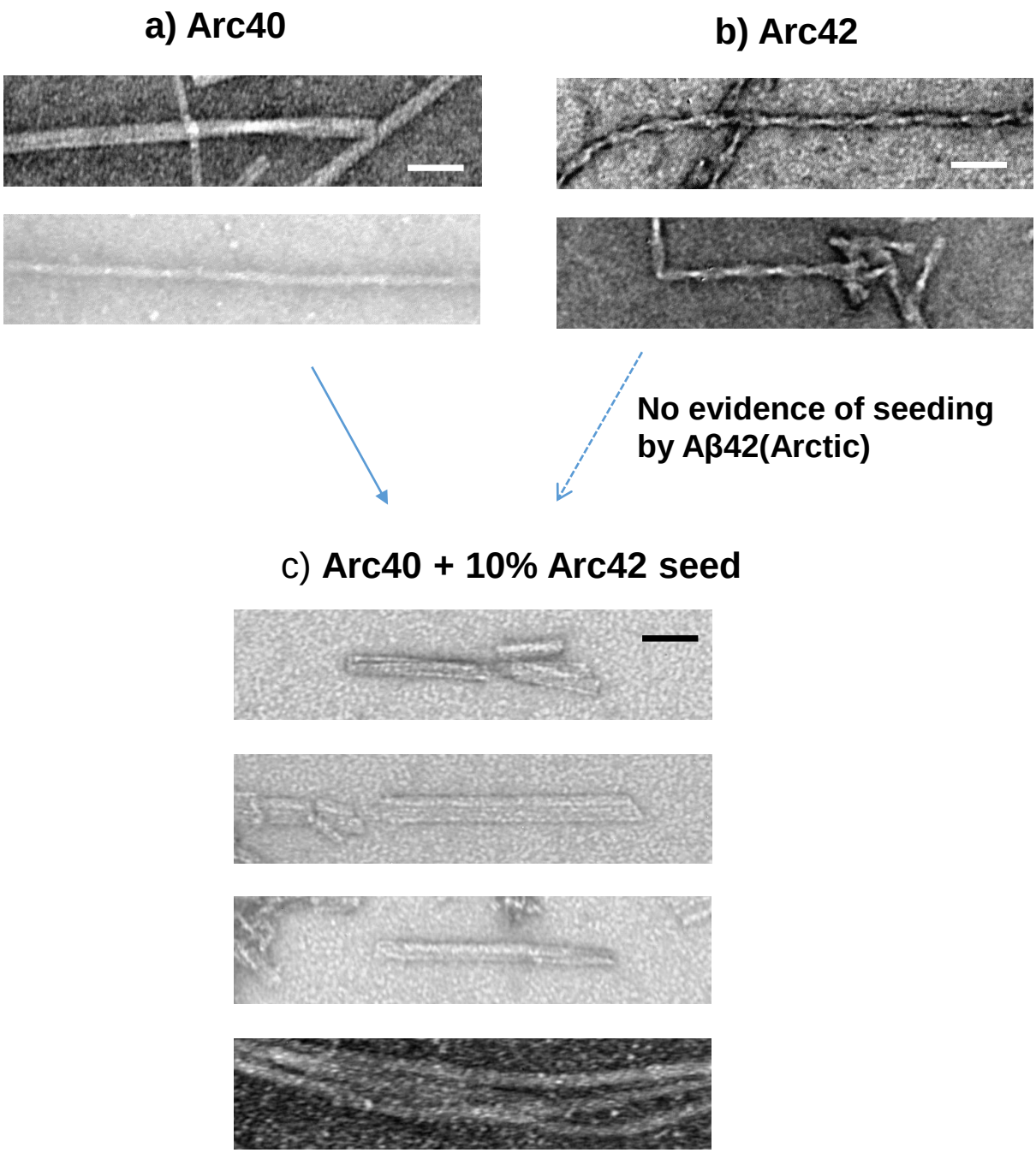

**Supplemental Figure S7: Cross-seeding of WildType Aβ40 fibrillization with seeds at different concentrations.** a,b,c) Lag time $t_{lag}$(a), half $t_{50}$ (b)and $k_{app}$ of WildType Aβ40 aggregation in the of fibril seeds: blue, WildType Aβ40; red, WildType Aβ42; purple, Italian Aβ40; and magenta, Arctic Aβ40. Symbols and error bars are the average and standard deviation of the four cross-seeding experiments.



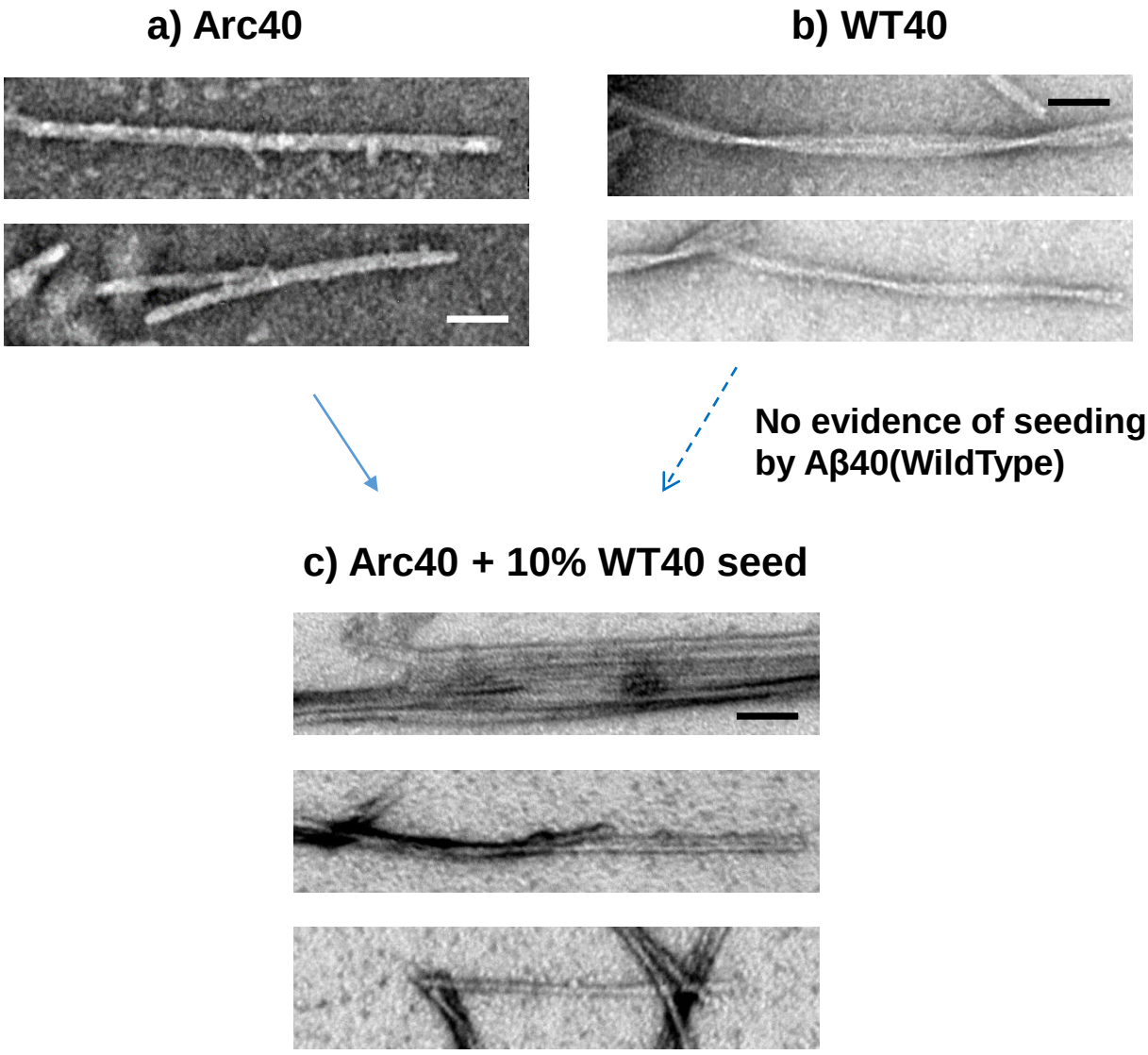

**Supplemental Figure S8**: **C-Amidated Aβ42** (a) Fibril growth kinetics of C-Amidated Aβ42. C-amidated Aβ42 (10 μM) was incubated with ThT (20 μM) in aqueous buffer containing NaCl (160 mM), HEPEs (30 mM), at pH 7.4. (b) TEM images of C-amidated Aβ42 fibril. Negatively stained TEM images generated from 10 μM C-amidated Aβ42 , 72 hrs incubation at pH 7.4, HEPES buffer (30 mM) and NaCl (160 mM).



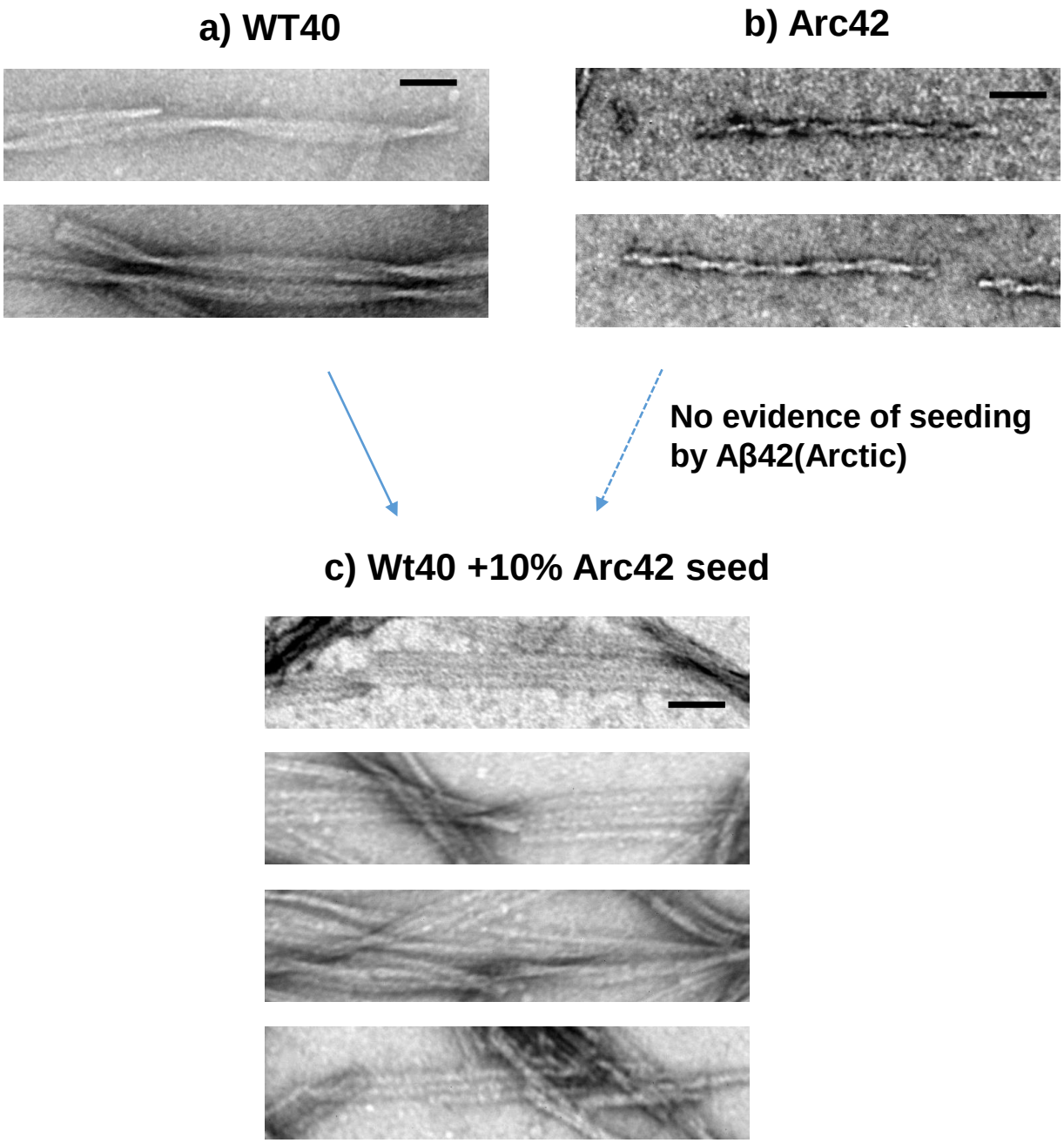

**Supplemental Figure S9:** TEM images of both seeded and unseeded Aβ42(WildType) and Aβ40(Arctic). a) Aβ40(Arctic); b) Aβ42(WildType); c) Aβ40(Arctic) with 10% Aβ42(WildType) fibril-seeds; d) Aβ42(WildType) with 10% Aβ40(Arctic) fibril-seeds. Aβ40(Arctic) with Aβ42(WildType) fibril-seeds added indicates cross-seeding as the Aβ42(WildType) fibril seed induces a marked twist in the otherwise untwisted Aβ40(Arctic) fibril isoform. While Aβ40(Arctic) fibril seeds cause a marked extension in the periodicity of the Aβ42(WildType) fibril twist from 80 nm to 125 nm. Scale Bar: 50 nm. (Note that Fig 7 is a simplified version of the data shown here, images are reshown here to aid direct comparison)




**Supplemental Figure S10:** TEM images of both seeded and unseeded Aβ40(Arctic). a) Aβ40(Arctic); b) Aβ42(Arctic); c) Aβ40(Arctic) with 10% Aβ42(Arctic) fibril-seeds. The morphology of Aβ40(Arctic) is unchanged by the presence of the highly twisted Aβ42(Arctic) fibril-seeds added, suggesting no cross-seeding. Scale Bar: 50 nm. (Note that the two images of unseeded Aβ40(Arctic) are reshown here to aid direct comparison with seeded fibrils and have already been shown in Fig S9a)




**Supplemental Figure S11:** TEM images of both seeded and unseeded Aβ40(Arctic), a) Aβ40(Arctic); b) Aβ40(WildType); c) Aβ40(Arctic) with 10% Aβ40(WildType) fibril-seeds. The morphology of Aβ40(Arctic) is unchanged by the more twisted presence of Aβ40(WildType) fibril-seeds added, suggesting no cross-seeding. Scale Bar: 50 nm. (Note that the two images of unseeded Aβ40(Arctic) are reshown here to aid direct comparison with seeded fibrils and have already been shown in Fig S9a)




**Supplemental Figure S12:** TEM images of both seeded and unseeded Aβ40(WildType) a) Aβ40(WildType); b) Aβ42(Arctic); c) Aβ40(WildType) with 10% Aβ42(Arctic) fibril-seeds. The morphology of Aβ40(WildType) is unchanged by the presence of the highly twisted Aβ42(Arctic) fibril-seeds added, suggesting no cross-seeding. Scale Bar: 50 nm.