Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary information

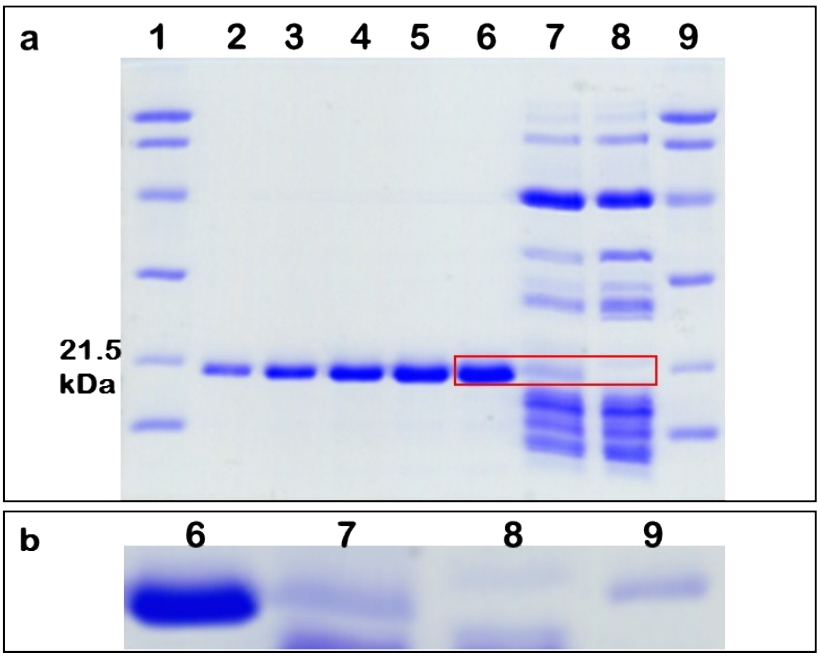

Figure S1. Expression of HGDP23T in a cell-free expression system; a) lanes 1 & 9 M_W marker; 2-6 HGD standards; 7 CFEM with P23T; 8, CFEM without P23T; (b) excised lines 6-9.

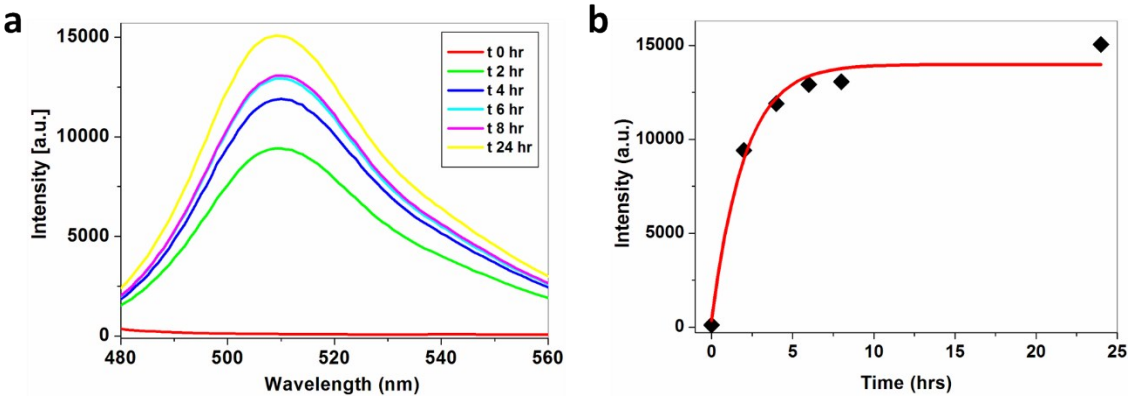

Figure S2. EmGFP fluorescence emission spectra (a) and concentration changes (b) during expression in a cell-free expression medium monitored by the increase in the fluorescence intensity over 24 hours. Protein expression is complete after 6 hours. The increase in fluorescence intensity at 24 hours is due to sample concentration because of evaporation.

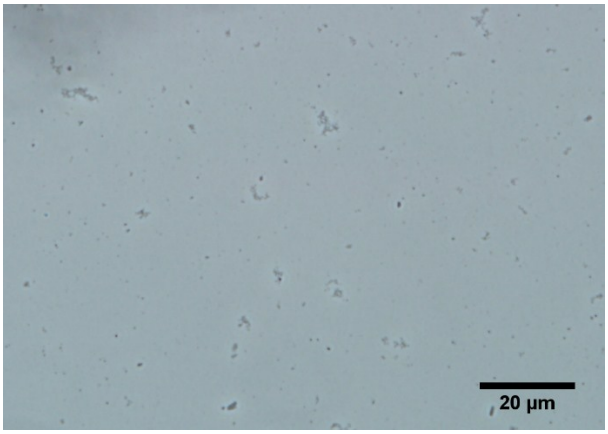

Figure S3. Phase contrast image of particles formed within the cell-free transcription-translation system (without the P23T HGD plasmid DNA) after 3 days of incubation at 37°C.

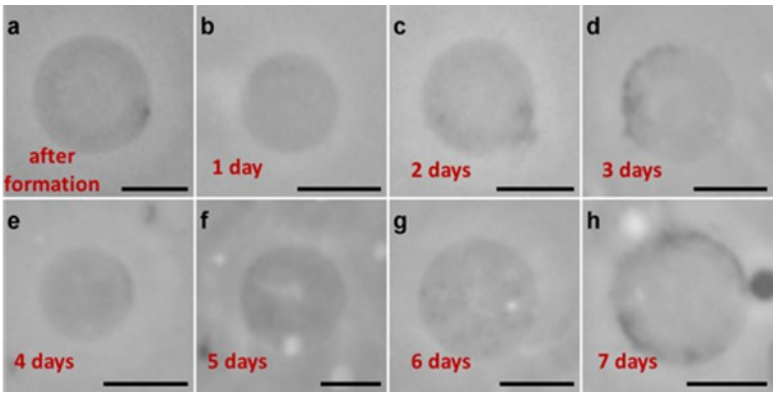

Figure S4. Phase contrast images of GUVs after expression of wild type HGD in the cell-free expression medium, (a) immediately after formation and then every 24 hours (b-h) (i.e. 8 days are shown here). Scale bar = 5 µm. Different (but representative) vesicles were imaged on each day.

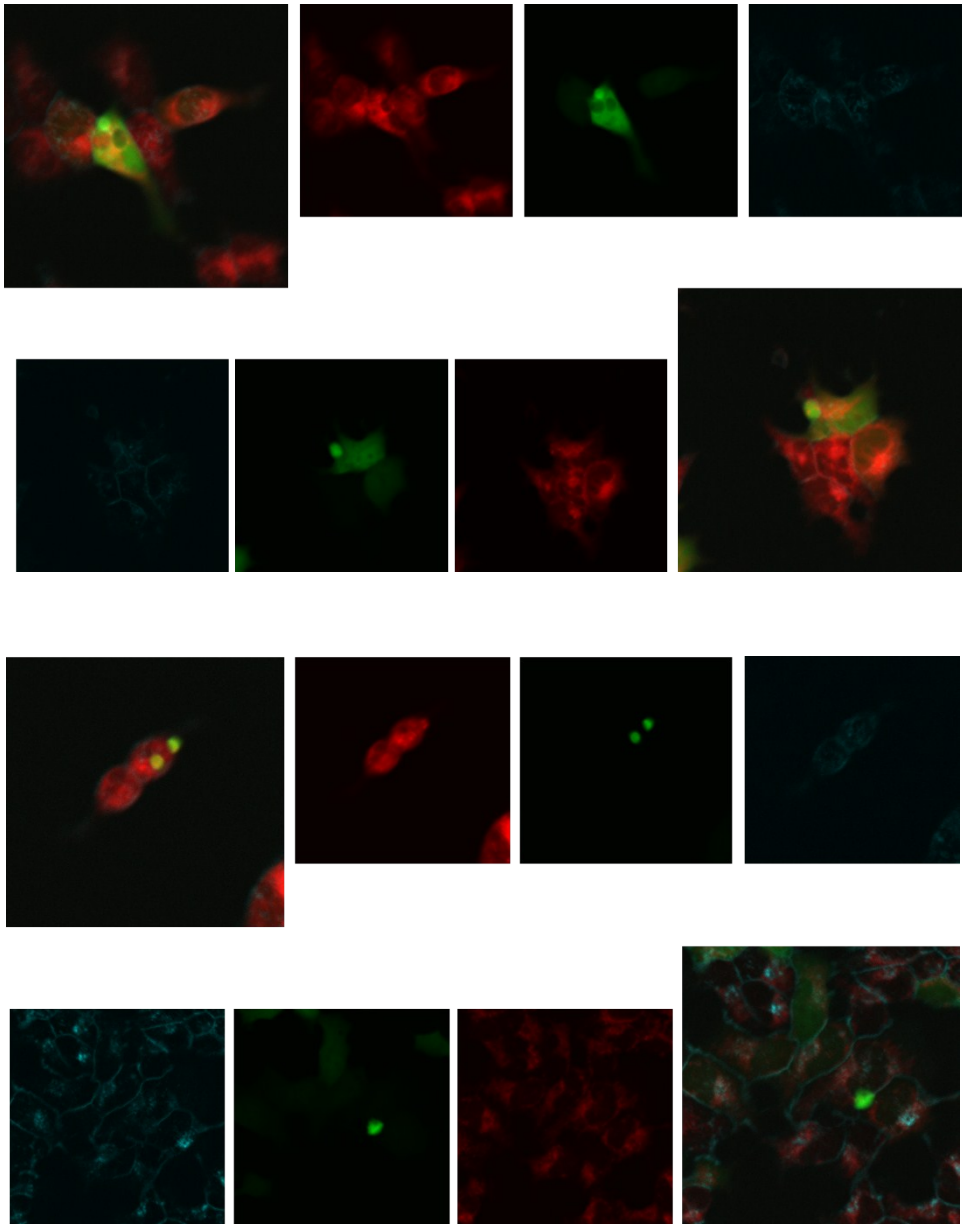

Fig S5: Confocal Microscopy images for HEK cells expressing the EmGFP-P23T fusion protein. Each of the four rows of images represents a individual region of the cell sheet – the larger image in each row is an overlay of the three smaller ones from the same row. Mitochondria are stained with MitoTracker Red CMXRos (red). Membranes are staied with Weat germ agglutinin Alexa Fluor 350 (blue). The EmGFP-P23T assemblies are green. We don't see localisation of the very large protein assemblies in the same regions as either membrane or mitochrondria. Our preliminary analysis indicates that the aggregates are most likely to be found in the cell cytoplasm.

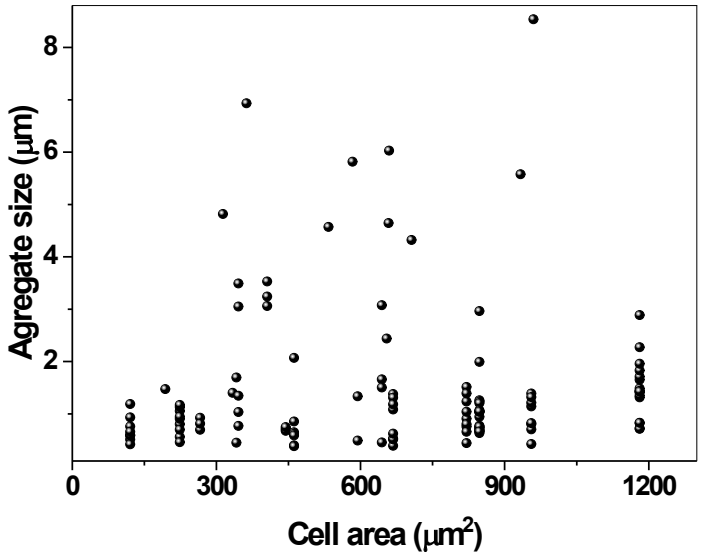

Fig S6: Range of EmGFP-P23T assembly sizes (diameter) formed in HEK cells for different HEK cells sizes. Since HEK cells are usually non-spherical, the 2 D cell area is used as an indicator of relative cell size. For comparision, the equivalent spherical diameter of a cell with an area of 150 μm is roughly 14μm, and for an area of 300 μm is roughly 19 μm. While smaller assemblies are observed in all cells, very large assemblies (larger than 2.5 μm) are only observe in cells larger than 300 μm.