

Figure S1. Double-immunostaining of undifferentiated, RA ad RA+BDNF SH-SY5Y with tyrosine hydroxylase (*red*) reveals expression of the protein in all treatment groups. Undifferentiated SH-SY5Y were stained as a secondary antibody control which revealed absence of unspecific staining. Nuclei were visualised using DAPI staining (*blue*). Scale bars represent 50 μm .

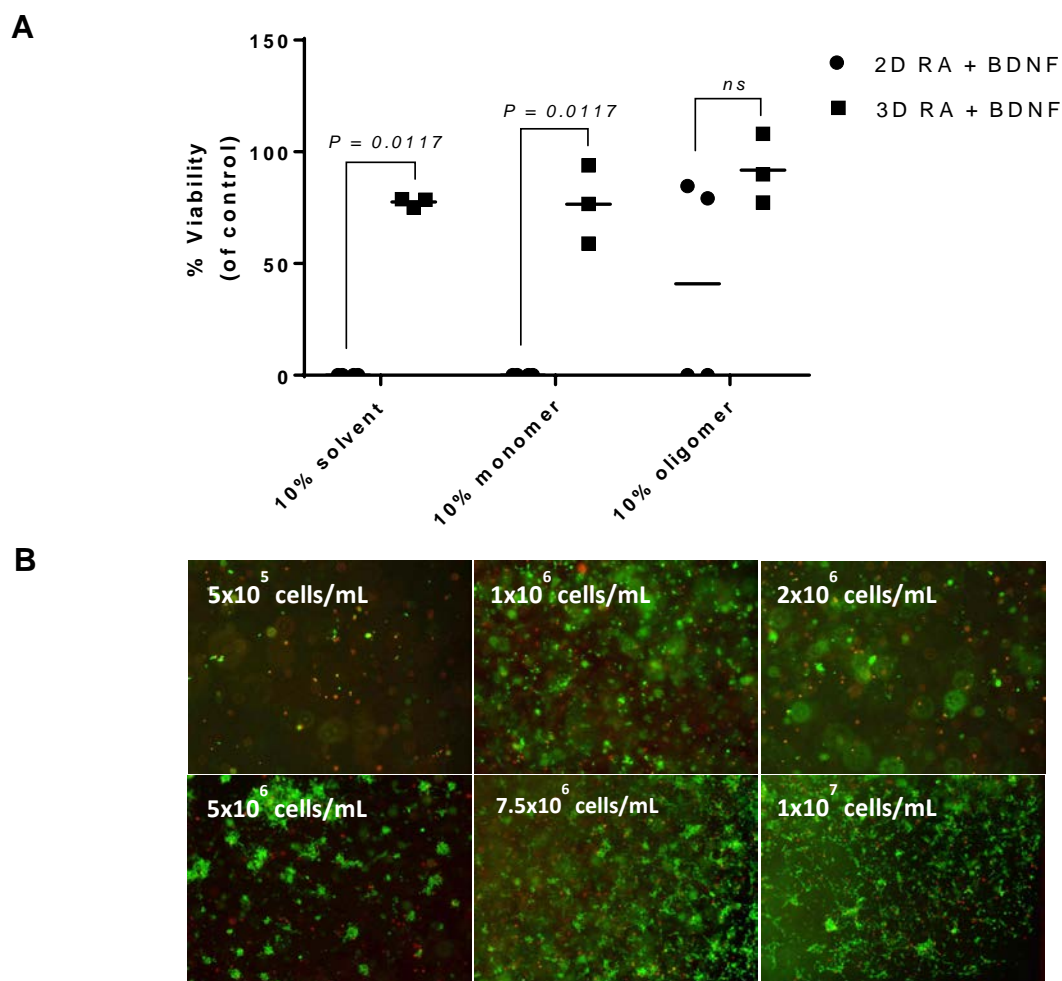


Figure S2. A. Alamar blue cell viability assay of 2D and 3D cultures exposed to monomeric and oligomeric α -syn and solvent controls. Treatment with seeding oligomers doesn't induce a significant amount of cell death in undifferentiated and RA-differentiated cultures. RA +BDNF SH-SY5Y are sensitive to solvent treatment, related to the cells state following differentiation, which is rescued by culturing RA-BDNF cell in a 3D format. All data points are present from at least three independent experiments, with mean shown as a straight line. Data presented as mean and SEM of at least 3 independent experiments. **B.** LIVE (*green*)/DEAD (*red*) staining reveals the distribution of cells throughout the 3D matrix.

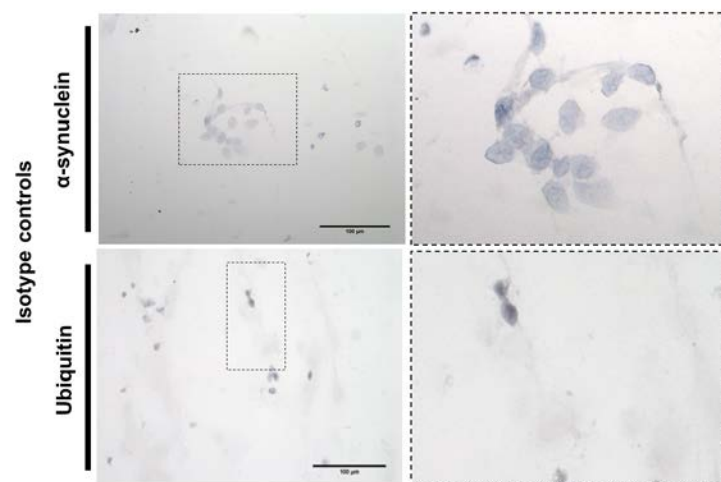


Figure S3. Immunohistochemistry isotype controls for α -syn and ubiquitin staining reveals the absence of non-specific background signal.

Figure number	Results of initial statistical test (Kruskal Wallis)	Results of post-hoc analysis					
Figure 1A	$P = <0.0001$	Undiff vs. RA $P = <0.0001$	Undiff vs. RA+BDNF $P = <0.0001$	RA vs. RA+BDNF $P = <0.0001$			
Figure 1B	$P = 0.0001$	Undiff vs. RA $P = 0.0022$	Undiff vs. RA+BDNF $P = 0.1046$	RA vs. RA+BDNF $P = 0.0015$			
Figure 1C	$P = <0.0001$	Undiff vs. RA $P = 0.0002$	Undiff vs. RA+BDNF $P = <0.0001$	RA vs. RA+BDNF $P = <0.999$			
Figure 1D	$P = 0.0001$	Undiff vs. RA $P = 0.0012$	Undiff vs. RA+BDNF $P = 0.919$	RA vs. RA+BDNF $P = 0.0005$			
Figure 1 E-DRD2	$P = 0.0321$	Undiff vs. RA $P = 0.5024$	Undiff vs. RA+BDNF $P = 0.0114$	RA vs. RA+BDNF $P = 0.0043$			
Figure 1 E-DAT	ns at: $P = 0.7217$	No further statistical analysis					
Figure 1 E-NES	ns at: $P = 0.1887$	No further statistical analysis					
Figure 1 E-MAP2	ns at: $P = 0.0575$	No further statistical analysis					
Figure 1 E-VMAT	ns at: $P = 0.1784$	No further statistical analysis					
Figure 1 E-TUBB3	ns at: $P = 0.5798$	No further statistical analysis					
Figure 2B	$P = <0.0001$	Undiff vs. RA $P = <0.0001$	Undiff vs. RA+BDNF $P = <0.0001$	RA vs. RA+BDNF $P = <0.0001$			
Figure 2C	$P = 0.0221$	Undiff vs. RA $P = >0.9999$	Undiff vs. RA+BDNF $P = 0.0223$	RA vs. RA+BDNF $P = 0.0223$			
Figure 1 E-DRD2	ns at: $P = 0.1413$	No further statistical analysis					
Figure 1 E-DAT	ns at: $P = 0.7125$	No further statistical analysis					
Figure 1 E-NES	ns at: $P = 0.3107$	No further statistical analysis					
Figure 1 E-MAP2	ns at: $P = 0.2477$	No further statistical analysis					
Figure 1 E-VMAT	ns at: $P = 0.0696$	No further statistical analysis					
Figure 1 E-TUBB3	ns at: $P = 0.0544$	No further statistical analysis					
Figure 4B	$P = 0.0329$	Media vs. Solvent $P = 0.6811$	Media vs. Monomer $P = 0.047$	Media vs. Oligomer $P = 0.0021$	Solvent vs. Monomer $P = 0.0242$	Solvent vs. Oligomer $P = 0.0012$	Monomer vs. Oligomer $P = 0.0656$
Figure 4C	$P = 0.0329$	Media vs. Solvent $P = 0.6811$	Media vs. Monomer $P = 0.047$	Media vs. Oligomer $P = 0.0021$	Solvent vs. Monomer $P = 0.0242$	Solvent vs. Oligomer $P = 0.0012$	Monomer vs. Oligomer $P = 0.0656$
Supplementary Figure 2A	Solvent- $P = 0.0193$	2D vs. 3D $P = 0.0117$					
Supplementary Figure 2A	Monomer- $P = 0.0193$	2D vs. 3D $P = 0.0117$					
Supplementary Figure 2A	Oligomer- $P = 0.1536$	No further statistical analysis					

Table S1. Results of all statistical analysis undertaken.