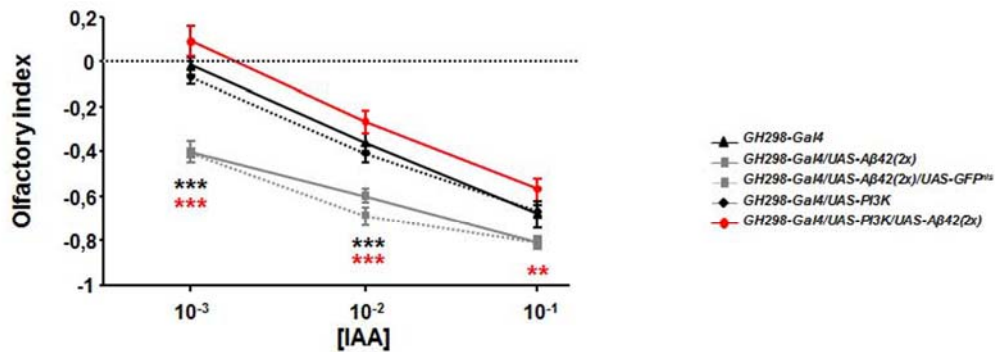


Supplemental Materials

Molecular Biology of the Cell

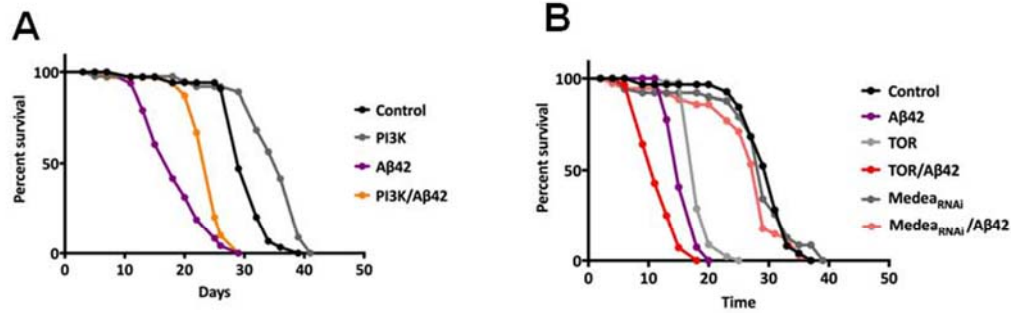
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Supplementary Data



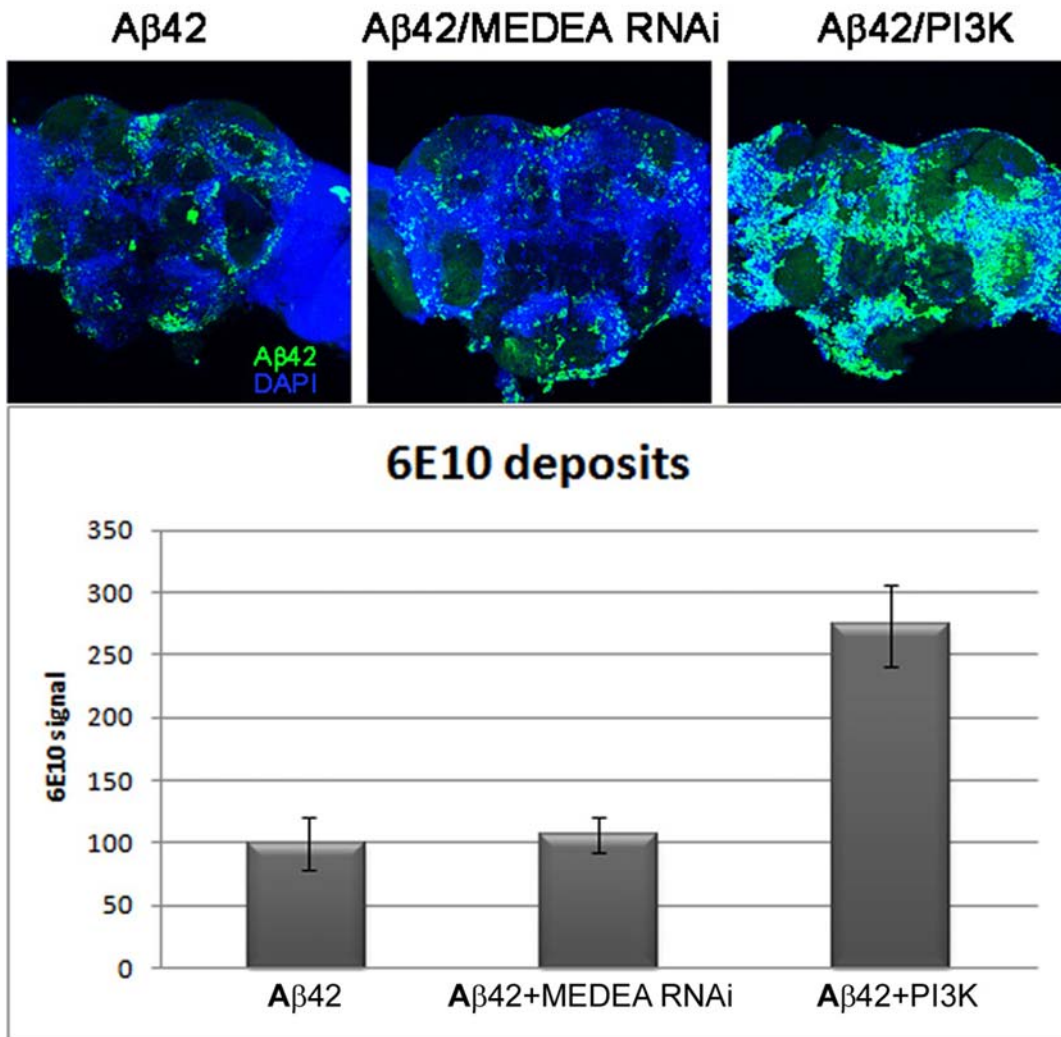
Suppl. Fig. S1

Supplementary Figure S1.- PI3K prevents Aβ42-induced olfactory perception defects to IAA. Adult 5-7 days aged flies were subjected to an olfactory assay for isoamyl acetate (IAA) with three concentrations (v/v). The expression of two different Aβ42 constructs in the *GH298-Gal4* domain (genotypes: *GH298-Gal4/UAS-Aβ42* and *GH298-Gal4/UAS-Aβ42/UAS-GFP^{nl5}*, full and dotted grey lines respectively) yields more repulsive responses to [10⁻³] and [10⁻²] compared to *GH298-Gal4* controls (full black line) and *GH298-Gal4/UAS-PI3K* flies (dotted black line). The inducible PI3K form (*UAS-PI3K^{92D}*) is used in this experiment. However, when PI3K and Aβ42 were co-expressed in GH298 neurons [genotype: *GH298-Gal4/UAS-PI3K/UAS-Aβ42(2X)*], the olfactory index returned to the normal profile (red line). Black asterisks: comparisons between *GH298-Gal4/UAS-Aβ42* and *GH298-* with respect to control. Red asterisks: comparisons between *GH298-Gal4/UAS-Aβ42* and *GH298-Gal4/UAS-PI3K/UAS-Aβ42* flies. **p<0.001; ***p<0.0001 (unpaired Student's *t* test with Welch's correction).



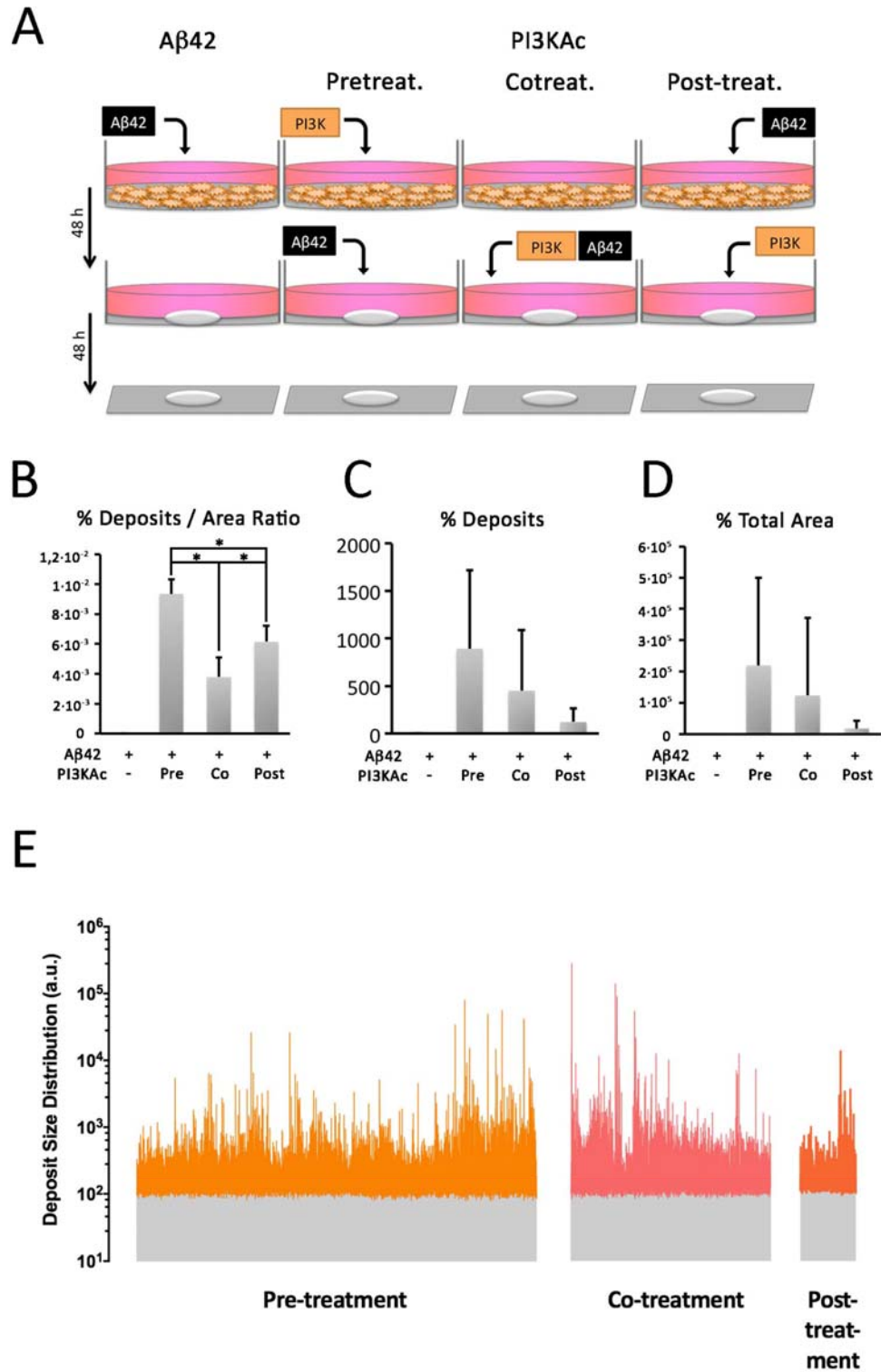
Suppl. Fig. S2

Supplementary Figure S2.- Aβ42-induced reduction of lifespan is prevented by PI3K synaptogenesis signaling.(A) Longevity profiles of color coded genotypes at 29°C. PI3K activation suppresses the lifespan reduction caused by Aβ42 to a large extent. Numerical data are shown in **Supp. Table S1.**(B) Equivalent profiles for other genotypes including factors that do not participate in the PI3K synaptogenic pathway, mTOR, or included in that signaling, Medea^{RNAi}. Note the full suppression by the later factor and the enhanced lifespan reduction caused by mTOR. Numerical data are shown in **Supp. Table S1.**



Suppl.Fig. S3

Supplementary Figure S3.-Medea downregulation does not increase Aβ42 deposits. Upper panels show confocal images of frontal views of 15 days old adult females expressing *UAS-Aβ42 (2X)* and *UAS-PI3K* or *UAS-Medea^{RNAi}* and stained with 6E10 antibody. Note the increased Aβ deposits in the PI3K brain, but not in the Medea^{RNAi}. Lower panel shows the quantification of the 6E10 signal in the whole stack of brain images in each genotype. A total of three brains per genotype were analyzed. Cell nuclei are marked by DAPI.



Suppl.Fig.S4

Supplementary Figure S4.- PI3K-induced increase in A β 42 aggregation can be reproduced outside the cell. (A) Cartoon representing the experimental design with SHT-SY5Y differentiated cells treated with A β 42 oligomers (36 μ g/ml) and PTD-PI3KAc peptide (50 μ g/ml) at different times of administration. Supernatants were stained with 6E10 antibody. Cells treated only with A β 42 oligomers were used as control. (B-E) Histograms representing percentage of deposits/area ratio (B), percentage of deposits (C), percentage total area (D), and deposit size distribution (E) of A β 42 and PI3K/A β 42 treated cells. Control cells treated only with A β 42 oligomers did not develop recognizable 6E10 positive deposits.

Over 4 cm / Paired T test (Two-tailed P value)				
Genotypes	Control	PI3K	Aβ42	PI3K/Aβ42
Control				
PI3K	0,6588			
Aβ42	< 0,0001****	< 0,0001****		
PI3K/Aβ42	0,0001****	< 0,0001****	0,0028**	

Bellow 4 cm / Paired T test (Two-tailed P value)				
Genotypes	Control	PI3K	Aβ42	PI3K/Aβ42
Control				
PI3K	0,2399			
Aβ42	0,4581	0,206		
PI3K/Aβ42	0,9078	0,7581	0,2074	

Bottom / Paired T test (Two-tailed P value)				
Genotypes	Control	PI3K	Aβ42	PI3K/Aβ42
Control				
PI3K	0,2775			
Aβ42	0,0194*	0,037*		
PI3K/Aβ42	0,136	0,5057	0,0379*	

Over 4 cm / Paired T test (Two-tailed P value)						
Genotypes	Control	Aβ42	mTOR	mTOR/Aβ42	Medea-RNAi	Medea-RNAi/Aβ42
Control						
Aβ42	0,031*					
mTOR	0,0082**					
mTOR/Aβ42	0,047*	0,1537	0,0549			
Medea-RNAi	0,0031**					
Medea-RNAi/Aβ42	0,0018**	0,0436*			0,3396	

Bellow 4 cm / Paired T test (Two-tailed P value)						
Genotypes	Control	Aβ42	mTOR	mTOR/Aβ42	Medea-RNAi	Medea-RNAi/Aβ42
Control						
Aβ42	0,4831					
mTOR	0,9284					
mTOR/Aβ42	0,7438	0,3288	0,7136			
Medea-RNAi	0,0835					
Medea-RNAi/Aβ42	0,2212	0,3384			0,4637	

Bottom / Paired T test (Two-tailed P value)						
Genotypes	Control	Aβ42	mTOR	mTOR/Aβ42	Medea-RNAi	Medea-RNAi/Aβ42
Control						
Aβ42	0,6361					
mTOR	0,0708					
mTOR/Aβ42	0,8737	0,0394*	0,0665			
Medea-RNAi	0,1166					
Medea-RNAi/Aβ42	0,1079	0,5755			0,6394	

Genotype	n	Median Surv	Maximum Surv	Mantel-Cox test
<i>Group VS Control</i>				
Control (UAS-LacZ/+)	30	29	36	
PI3K (UAS-PI3K caax/+)	34	36 (+24%)	39 (+8%)	p < 0,0001
Aβ42 (UAS-2xAb42/+)	48	18 (-38%)	24 (-33%)	p < 0,0001
PI3K/Aβ42 (UAS-PI3K caax/UAS-2xAb42)	30	25 (-14%)	27 (-25%)	p < 0,0001
<i>PI3K/Aβ42 VS Aβ42</i>				
Aβ42 (UAS-2xAb42/+)	48	18	24	
PI3K/Aβ42 (UAS-PI3K caax/UAS-2xAb42)	30	25 (+38%)	27 (+12%)	p < 0,0001

Genotype	n	Median Surv	Maximum Surv	Mantel-Cox test
<i>Group VS Control</i>				
Control (UAS-LacZ/+)	25	31	35	
Aβ42 (UAS-2xAb42/+)	27	15 (-52%)	20 (-43%)	p < 0,0001
mTOR (UAS-mTOR/+)	45	18 (-42%)	23 (-34%)	p < 0,0001
mTOR/Aβ42 (UAS-mTOR/UAS-2xAb42)	28	11 (-64%)	15 (-57%)	p < 0,0001
Med RNAi (UAS-MedeaRNAi/+)	46	29 (-6%)	37 (+6%)	p = 0,9609
Med RNAi/Aβ42 (UAS-Medea RNAi/UAS-2xAb42)	34	29 (-6%)	35 (+)	p = 0,1482
<i>PI3K/Aβ42 VS Aβ42</i>				
Aβ42 (UAS-2xAb42)	27	15	20	
mTOR/Aβ42 (UAS-mTOR/UAS-2xAb42)	28	11 (-39%)	15 (-25%)	p < 0,0001
Med RNAi/Aβ42 (UAS-Medea RNAi/UAS-2xAb42)	34	29 (+61%)	35 (+75%)	p < 0,0001

Suppl. Table S1

Supplementary Table S1.-Data from climbing and lifespan assays with the corresponding statistical tests. Climbing assay performance is color coded as in main Figures 2 and 6.